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AN EXPERIMENTAL STUDY ON THE PROBLEM OF LYMPH NODE REGENERATION

BY

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THESIS

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AN EXPERIMENTAL STUDY OF LYMPH NODE REGENERATION IN RABBITS [1]

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TWENTY-THREE FIGURES

I. INTRODUCTION

The question of the regeneration of lymph nodes following their removal has been much disputed, despite the numerous papers on this subject. Critical analysis, however, of the past researches reveals several important and interesting points. A variety of laboratory animals including rabbits, dogs, guinea pigs, mice and rats have been used, with minimal attention given to the age of such experimental material. The method of node removal as well as the length of the post-operative period varied greatly. Added to this confusion is the fact that the site of removal was not uniform in the different investigations. Finally the term "regeneration" has been employed with laxity sufficient to arouse misconceptions. The writer feels that the distinction between regeneration and repair is not always drawn clearly. Regeneration, as here defined, implies the new formation of the removed part or organ by progressive differentiation of primitive or undifferentiated tissues. Repair, on the other hand, implies restoration by the proliferation of the residual portion of the organ in which the tissues are already differentiated. Hence a careful consideration of the experimental conditions, under which

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regeneration or lack of regeneration occurred, seems a prerequisite for correct interpretation. This point, unfortunately, is often overlooked by authors of current texts and reviews.

The present investigation, using the rabbit as the experimental animal, was undertaken with the above considerations in mind. Though at the outset the proposed plan was to study the regeneration of lymph nodes in a large number of adult rabbits, the writer — proceeding on the general premise that the younger the organism the more vigorous the regeneration of removed parts (if regeneration is possible at all) — first employed groups of young rabbits, ranging from birth to 32 days, on a trial basis. The initial results indicated, however, that this presumption was relatively erroneous, for in the older litters of that range the degree of regeneration appeared to be more complete than in the younger ones. This observation emphasized that the age of the organism is definitely linked with the regenerative capacity of lymph nodes. Consequently the course of the experiment was altered to extend the study of lymph node regeneration in rabbits over the entire range, from the period of the newborn through adult life to senility.

The writer expresses gratitude to Professor Otto F. Kampmeier whose inspiring encouragement, aside from his generous advice and guidance made possible the undertaking and completion of this work. Thanks are due also to Mr. Arthur Hesse, Director of the Animal Hospital for his efforts in the procurement of the animals during the adverse wartime period.

II. REVIEW OF LITERATURE

Bayer (1885) extirpated the axillary lymph nodes in a small number of dogs ranging from several weeks of age to 1½ years. Allowing a minimum of 3 weeks for the post-operative period, he found what appeared to be newly-formed nodes at the site of extirpation. He concluded that regeneration was brought about by the direct transformation of adipose tissue into lymphatic tissue. Lymph stasis was believed to be the prime

factor in the initiation of the regenerative process. Bacialli (1886) resected only a portion of a popliteal node of dogs and observed regeneration from the residual part. He, too, held that neoformation occurred by direct transformation of the perinodal fat tissue. Delius (1888) and Ribbert (1889), using dogs and rabbits respectively, obtained similar results after partial removal of a node. Delius, however, maintained that the local "fixed tissue cells" gave rise to lymphatic tissue, while Ribbert regarded the lymphatic endothelium as the chief source. Contrary to Bayer, Ribbert ascribed to local irritations the stimulus for regeneration; he arrived at this conclusion after finding optimum regeneration in animals in which he had inserted a piece of sterile sponge at the site of extirpation.

The first opposition to the positive findings of the earlier authors was registered by Heuter ('04), who did not see any regeneration of parenchymatous tissue following partial removal of individual cervical nodes in dogs. He also made an interesting observation, that of a residual node being divided by scar tissue into islands which in histologic sections looked like newly-formed nodules. Meyer ('06) found no indication of regeneration after excision of mesenteric and popliteal lymph nodes in dogs. In 1 animal, however, he saw a small node at the site of removal, but because of the presence of a well-formed capsule this was considered as a residual node. According to Vecchi ('11), complete removal of the popliteal, the retrodeltoid, or the cervical nodes in rabbits, dogs and guinea pigs resulted only in their replacement by scar tissue. In several of the rabbits the entire perinodal tissue was removed along with the node, a procedure which was criticized vigorously by subsequent workers. Vecchi obtained negative results also after resection, that is, partial excision, of the popliteal and retrodeltoid nodes in rabbits and dogs.

Groot ('12) observed positive regeneration after extirpation of the axillary nodes in mice and guinea pigs. Subcutaneous injections of sterile clay distal to the site of extirpation were made to provide the stimulus for regenera-

tion. In mice he saw islands of unorganized lymphatic tissue interspersed in fat without the formation of true nodes. In the majority of the guinea pigs complete regeneration of small nodes occurred. The negative results were attributed by Groot to post-operative infections. He was in accord with Bayer and several of the early investigators who stated that the new lymphatic tissue arose from the transformation of adipose tissue.

Baum ('26), in a study which dealt not with the regeneration of lymph nodes but with the restoration of lymphatic channels, removed the popliteal, submandibular, or the superficial inguinal nodes of young and adult dogs. In all the young animals, except one, he examined the effect after a post-operative period less than 14 days. Only in the 1 animal excepted, which had a post-operative period of 70 days, was there evidence of node regeneration. The negative findings in the other young dogs are not surprising, for in the light of the present writer's observations 14 days appear to be entirely too short a period to permit much progress towards regeneration. In none of the adult dogs did Baum see any signs of neoformation despite longer post-operative periods.

After total excision of the axillary nodes in adult guinea pigs, Auché ('30) observed their regeneration in three-fourths of the animals. On the basis of his histologic findings, he stated that new lymphatic tissue developed from certain potent cells in the areolar tissue and between the small fat lobules. Although Auché gave no details in regard to the ages of his experimental animals, he maintained that youth and mild irritations were factors conducive to regeneration, while senility, pregnancy, and severe local or systemic infections had the opposite effect. Furthermore, he was the first to express the opinion that lymph nodes did not form in any random locale but only where such an organ had been removed previously or, to use his own words, "in sites of node suppression."

According to Moriconi ('32), partial excision of abdominal lymph nodes in dogs, or linear incisions into them, resulted

merely in hemorrhagic infiltration with subsequent replacement by scar-tissue.

About 2 weeks after the total removal of the popliteal node in the rabbit, Wasa ('32) found lymphatic accumulations between the plexus of reestablished lymph channels.

Total excision of the popliteal node in a large number of adult rabbits by Ottaviani and Cavalli ('33) failed to produce regeneration other than the reestablishment of the severed lymph channels. These investigators ('34) reported negative findings also upon removing the submaxillary and cervical nodes in adult dogs. Their results were determined without resorting to microscopic studies.

A low incidence of regeneration after extirpation of the axillary, inguinal or popliteal nodes in adult guinea pigs was reported by Teneff ('34). He concluded that regeneration was possible either by hyperplasia of "rudimentary nodes," or by the transformation of elements in the loose connective tissue. Papillian and Russu ('34) cut out the mesenteric nodes in dogs and observed the formation of scattered lymphatic nodules in the surrounding mesenteric tissues.

Complete regeneration of the popliteal lymph node occurred in rabbits, according to Okubo ('35), who carefully excised its parenchyma but left the capsule and vascular connections in situ with a minimum of injury. Takeshita ('36), on the other hand, failed to find any indications of neoformation after extirpation of the popliteal node in adult rabbits, but the reestablishment of lymph channels took place after a period of about 3 weeks. In another set of experiments ('37), again on adult rabbits, Takeshita removed only one-half of the popliteal node. The residual segment of the node became well-encapsulated without hyperplasia. Employing Okubo's technique, Horii and Sakai ('37) "shelled out" the parenchyma from the popliteal node of rabbits and obtained neoformation of a functional node from the residual capsule.

The complete removal of the popliteal node in a large number of adult rabbits by Rouvière and Valette ('37) failed to yield indications of its regeneration. A second experimental

group, in which about four-fifths of each node was excised, gave a small degree of regeneration — we would say, repair — from the residual parenchyma in about a third of the animals.

Sanders and Florey ('40) extirpated most of the somatic and visceral lymph nodes of young rats and adult rabbits in order to ascertain the changes of weight in the remaining lymphatic tissue. They did not find any regenerated nodes in the areas of operation and concluded that positive experimental results were due probably to faulty or incomplete removal.

III. THE PRESENT INVESTIGATION

1. Material

The experimental material consisted of a total of 152 rabbits ranging in age from 3 days to approximately 3½ years. By special arrangement with reliable commercial rabbit farms, weanlings and young adults were obtained with exact age and litter histories. In order to care satisfactorily for animals younger than 45 days, pregnant mothers were secured and allowed to bear litters in the Animal Hospital of the University of Illinois, College of Medicine. All animals were maintained under proper conditions in this hospital throughout the length of the investigation. The adults were fed an adequate ration (Rockland Rabbit Pellets), while the younger animals after weaning were given necessary dietary supplements.

Table 1 shows the disposal of the 152 rabbits in 4 separate experimental groups. The number of animals, the age range, and the lengths of the post-operative periods are stated for each group. Group I, consisting of 31 young animals (3–32 days old), with a post-operative period of 5 to 60 days, represents the material used in the initial experimentation. The right popliteal node of each rabbit was extirpated and the residual perinodal tissue studied at the end of the post-operative period, at which time the left popliteal node also was removed to serve as a specimen for comparison. The experience and data gained from such initial work enabled the

writer to proceed with a definite standardized technique throughout the remainder of the investigation. Group II includes 83 young and near-adult rabbits (27–153 days old), with post-operative periods from 8 to 102 days. Since results of the preceding group demonstrated that a control specimen from each animal was unnecessary, the popliteal nodes were

TABLE 1

Grouping of experimental animals.

GROUP	NO. OF ANIMALS	AGE RANGE	POST-OPERACIVE PERIOD
I. Initial group of young animals. Right popliteal node removed. Left popliteal node used as control. Total = 31 rabbits, 31 nodes removed.	2	3 days	30– 49 days
	7	6– 8 days	5– 21 days
	16	14– 17 days	29– 60 days
	6	26– 32 days	19– 33 days
II. Young animals. Bilateral removal of popliteal nodes. Total = 83 rabbits, 166 nodes removed.	25	27– 31 days	8– 47 days
	12	45 days	30– 32 days
	15	60 days	18– 32 days
	15	90– 96 days	31–102 days
	16	120–153 days	30– 32 days
III. Adult and senile animals. Total = 16 rabbits, 29 nodes removed.	10	8– 15 months	68–330 days
	6	$2\frac{1}{2}$– $3\frac{1}{2}$ years	31– 91 days
IV. Young animals. This group served for the study of the mode of regeneration. Bilateral removal of popliteal nodes. Total = 22 rabbits, 44 nodes removed.	10	45– 46 days	7– 28 days
	12	44– 46 days	14– 22 days

removed from both right and left hind legs. Hence this group supplied the 166 extirpations for the major part of the study of regenerative activity. Group III consists of 10 adults (8–15 months old) and 6 senile animals (approximately $2\frac{1}{2}$–$3\frac{1}{2}$ years old). The post-operative periods are 68 to 330 days and 31 to 91 days, respectively. Bilateral extirpation of the popliteal

nodes was performed in 7 of the adults and in all 6 of the old-age rabbits, making a total of 29 cases studied.

After the results from the above groups were analyzed, the mode of regeneration of lymph nodes remained to be studied by examining their developmental stages according to chronologic sequence. The material for this final division of the problem is listed in Group IV, which contains 22 young rabbits (44–46 days old). Choosing this particular age-group was based on the figures obtained in Groups I, II and III which indicated that regeneration both as to incidence and quality was at an optimum in animals approximately 1½ months old. The popliteal nodes were removed bilaterally, giving a total of 44 cases. The short post-operative periods of 7 to 28 days were assigned with a view towards ascertaining as closely as possible the critical period of neoformation.

2. The anatomy of the popliteal space and its contents in the adult rabbit

For the sake of orientation and clarity the gross morphology of the popliteal region of the rabbit needs to be considered briefly.

The popliteal space is bounded medially by the semimembranosus and adductor magnus, laterally by the biceps femoris, above by the convergence of these 3 muscles and below by the 2 convex bellies of the gastrocnemius. Superficially the lozenge-shaped potential space is covered by the crural fascia. A small superficial vein (anastomotic branch) runs diagonally over this fascia and connects the great saphenous vein below with the sciatic vein above (fig. 1 A). The lymph node lies superficial and distal to the center of the space and is always well embedded in adipose tissue. Its hilar pole points deeply (towards the knee-joint) and proximad (fig. 1 B). The artery supplying the node arises from the small saphenous artery near the heads of the gastrocnemius muscle. Before entering the hilum, this vessel furnishes large branches to the hamstring muscles and several small but constant twigs to the perinodal fat and the overlying superficial

fascia. The arterial branches are accompanied by corresponding venous tributaries. A small vein, which has no arterial counterpart, however, connects the superficial anastomotic branch (vide supra) with the nodal vein. The afferent lymphatics enter along the periphery of the node, while the efferent lymphatic (sometimes 2) leaves the hilum and courses

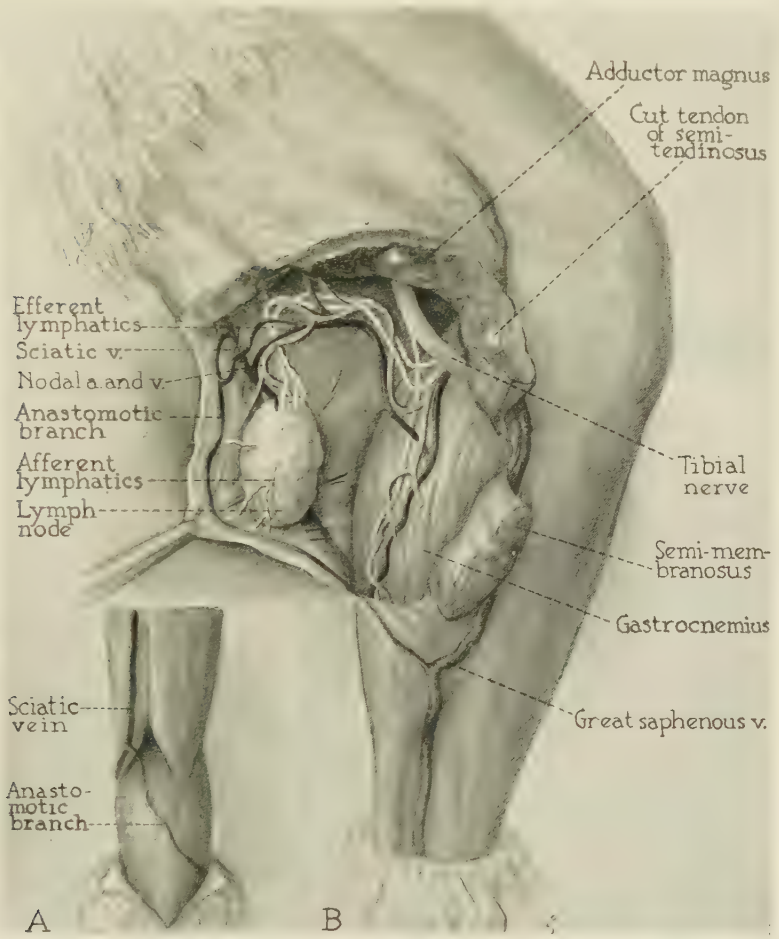

Fig. 1 *A*. Popliteal surface of the left hind limb of an adult rabbit seen from behind. The skin has been reflected to show the course of the anastomotic vein. × 1. *B*. Contents of the left popliteal space of an adult rabbit exposed by a medial dissection. × 1.2.

deep along the nodal artery and vein towards the short saphenous vessels. There that lymphatic joins with afferents ascending from the posterolateral surface of the leg along the short saphenous vein, and the combined tract now passes proximally to meet the deep popliteal lymphatics.

The size of the popliteal node is variable, although in the average adult animal it measures about 8×12 mm prior to fixation. The form of the organ, while usually ovoid, may be kidney-shaped, pea-shaped or may even be conical with the hilum located at its base. Rarely the node may be bilobed unevenly but an independent supernumerary node was never encountered in the course of this investigation.

3. Methods

A. Surgical technique. The fur around the popliteal area is removed cleanly by trimming and by an application of sulphide depilatory. To minimize the length of time under anaesthesia, ether is not given until after these preparatory steps are completed.

Sterilized instruments are used and the operation done in a clean, draft-free room. With the leg of the animal held firmly with one hand, a central, straight proximo-distal incision about 2 cm long is made in the skin of the popliteal region. Slight pressure applied from the outside on the lateral and medial walls of the popliteal space evaginates the soft areolo-adipose tissue containing the node. The differentiation of the lymph node from the surrounding tissue requires considerable experience. When viewed under good light and with the aid of a hand lens, the node appears as a pinkish-white, opaque body, whereas the adipose tissue in which it is embedded is translucent and quite refractile or glittering. The excess perinodal fat is separated carefully from the node with forceps and iridectomy scissors. When the hilum is approached, and the node is taken out by transecting the nodal artery, vein and the efferent lymph trunk. As a precautionary measure, a final examination with the hand lens is made to detect any possible remnants of the node. Ligation of the stump of the nodal artery

is not necessary. The incision is closed with 1 or 2 linen sutures. In bilateral extirpation the node of the opposite limb is removed in an identical manner.

In about 7.5% of the cases hematomas developed at the site of operation after the animal emerged from the anaesthesia and moved around in the cage. This did not seem to be a serious detriment to regeneration as evidenced by the later results.

The excised node is fixed in 10% neutral formalin. From each group of animals several nodes are prepared for histologic study in a manner described below. Such specimens serve as control material, making it possible not only to verify the completeness of their removal, but also to establish their morphological state at the different periods of life.

B. Autopsy. One hour before sacrifice, dilute India ink is injected subcutaneously (0.2 cm^3, 1 part ink to 3 parts physiologic saline) into the dorsal and plantar surfaces of the foot to determine the flow of lymph into the area from which the node had been removed. After death of the animal the entire content of the popliteal space is dissected out carefully, observed with hand lens and fixed in formalin.

C. Preparation of tissue for microscopic study. The fixed tissues are washed, dehydrated, cleared, embedded in paraffin and sectioned serially at 8 to 10 μ. Hematoxylin and eosin is the standard stain used. Central sections of the original and the regenerated nodes were measured with a filar micrometer. Hence, values appearing in the subsequent paragraphs represent dimensions of these structures after completion of histologic technique.

4. The morphogenesis and the retrogression of the popliteal node in the rabbit

In any study on regeneration, a preliminary knowledge both of the normal development and the senile atrophy of the organ which is to be the subject of experiment is necessary. Accordingly a histologic collection of a series of popliteal lymph nodes of rabbits from the earliest stage to senility was

prepared. The material representing the steps in intrauterine development (birth occurring on the 30th day after conception) was obtained from a limited number of normal fetuses of accurately determined ages. The remainder of the series consisted of representatives selected from a large number of normal nodes that accumulated throughout the experiments.

The anlage of the popliteal node is not apparent in fetuses of 17 days. In the next fetuses that were examined, those of 21 days, the anlage is an oval structure, approximately 0.3×0.6 mm, and surrounded by a wide sinus in the loose mesenchymal tissue of the popliteal space. The chief blood vascular supply to the anlage passes through a broad bridge of tissue at the deep pole, while additional vessels of capillary dimensions traverse most of the numerous finer strands found along the periphery. Lymph channels, arising in the subcutaneous region of the leg, converge towards the anlage and join the peripheral sinus at its superficial extremity. Similar channels leave this sinus at the opposite pole and run deep into the popliteal tissue, in close relation to the stems of the principal nodal blood vessels. The anlage of the node consists of a relatively solid cluster of typical mesenchymal cells with large pale nuclei and abundant eosinophilic cytoplasm. Located between their processes are widely scattered smaller cells resembling hemocytoblasts with round chromatic nuclei and scanty basophilic cytoplasm. Mitotic figures are met more frequently in them than in the larger mesenchymal cells.

In fetuses of 26 days the anlage measures approximately 0.7 mm in diameter and is broken up into irregular cords by numerous spaces which are in continuity with the peripheral sinus. The intervening spaces are more numerous towards the center so that already at this stage the developing node presents an outer dense and an inner diffuse portion. The mesenchymal cords and islands appear less dense than in the anlage of the 21-day fetus, making the interlacing cytoplasmic processes more easily discernible. Occasionally, small cells resembling lymphocytes are interspersed here and there within the cords and islands.

In the newborn animal the developing popliteal node measures approximately 0.6 × 1.0 mm and, for the first time in the writer's series, shows definite lymphopoietic centers. The latter are made up of dense nodules of lymphocytes and are limited to the peripheral zone of the node. Cords and islands of mesenchymal cells, separated by irregular sinusoidal channels, still make up the inner zone of the future medulla. No lymphocytes are found here except those that lie free in the sinusoids. In the 8-day animal the node has almost doubled in size to approximately 1 × 1.3 mm. No significant changes from the previous stage are recognizable in the cortex except for the numerical increase of the primary nodules. Small lymphopoietic centers, however, appear in the zone of the medullary cords lying adjacent to the inner rim of the cortex.

In 14-day animals the node has enlarged to about 1.7 × 2 mm. A thin but definite capsule of fibrous tissue surrounds it and sends trabecular processes centrally. More extensive lymphopoietic activity of the parenchyma is evidenced by the appearance of small lymphatic masses throughout the entire medulla continuing even into the region of the hilum. The node now possesses all of the structural components of a definitive organ and differs essentially from the latter only in size. The subsequent changes in the developmental progress of the lymph node are those of quantitative growth. From measurements of several specimens in each category of age, the node is about 2.8 × 4 mm at 30 days, 3.5 × 5 mm at 45 days, and 5 × 8 mm at 90 days. At 150 days the adult dimensions of approximately 6 × 11 mm are attained.

Marked histologic changes indicative of senility appear in the nodes taken from animals of 2½ to 3½ years of age. The specimens are characterized by small patches of fat in the reticulum of the medulla of the hilar region which extend into their central portion (fig. 3). The white fibrous component of the stroma also shows a definite increase, as evidenced by the thickened capsule and the trabeculae. The average size of the nodes remains within the limits of the adult dimensions, although greater individual variations are encountered.

Jordan and Looper ('27) stated that senile retrogression in the peripheral somatic lymph nodes of rabbits leads to a complete loss of normal structures and to cessation of function. Histological examination of the extirpated popliteal nodes of senile rabbits in the present investigation failed to confirm this assertion. In fact, quite the contrary was observed, when in 6 of the old rabbits the writer attempted to "activate" the popliteal node of one side prior to excision in order to test its capacity of response to stimulation. After the right popliteal node was removed for subsequent comparison, 0.2 cm^3 of typhoid vaccine was injected subcutaneously into the left leg 48 hours before extirpation. Figure 4 illustrates the result of this treatment. Aside from the evident increase in size when compared with the control (fig. 3), several significant changes in structure occurred throughout the node, chief of which was active hyperplasia of the lymphopoietic tissue. The capsule over the areas of hyperplasia is not only thin but exhibits numerous out-pocketings due to the formation of new lymphopoietic centers. Secondary nodules are much more numerous in the cortex than in the control. The medulla also contains active centers of cellular proliferation which appear as 3 large condensations (*lc*) in figure 4. The intervening medullary tissue is seen to possess an increased number of reticular cells and to resemble the comparable area of young adult specimens. Although patches of fat are still found near the large vessels of the hilar region, these are altered by the presence of large cells, resembling reticular cells, between the microscopic adipose lobules.

5. Regeneration of the popliteal node in the rabbit

A. Group I of young animals for initial experiment. Extirpation of the right popliteal lymph node. Table 2 summarizes the experimental data of this group of 31 animals, 3–32 days old. Under "node regeneration" both the gross observations of the specimens at the time of autopsy and the results of its subsequent microscopic examinations are given. The former are expressed either as (+), that is, the presence of a grossly

visible node, or as (—), failure of detecting any structure resembling a regenerated node in the dissected popliteal tissue. The symbols preceding the statement of the results of microscopic examinations are to be interpreted as follows: the

TABLE 2

Summary of results. Group I — initial group.

ANIMAL NO.	AGE, DAYS	POST-OP. PERIOD, DAYS	NODE REGENERATION	
			Gross	Microscopic
3A	3	30	—	∓ scattered lympho-reticular islands
1E	3	49	—	+ poorly-differentiated node, 0.8 × 1 mm
4A	6	5	—	∓ lympho-retic. islands in granulation tissue
4B	6	9	—	∓ scattered lympho-reticular islands
4C	6	13	—	∓ scattered lympho-reticular islands
4D	6	17	—	± lymphatic nodule, 0.3 × 0.5 mm
4E	6	21	—	∓ scattered lympho-reticular islands
4F	6	14x	—	∓ scattered lympho-reticular islands
3C	8	21x	—	∓ scattered lympho-reticular islands
7A	14	30	—	∓ scattered lympho-reticular islands
7B	14	30	—	+ poorly-differentiated node, 0.6 mm diam.
7C	14	30	—	∓ scattered lympho-reticular islands
8A	15	30*	—	± lymphatic nodule, 0.3 × 0.5 mm
9A	14	29	—	∓ scattered lympho-reticular islands
9B	14	49	—	area of experiment lost in preparation
9C	14	49	—	+ well-differentiated node, 1.2 × 2.3 mm
9D	14	29	—	area of experiment lost in preparation
9E	14	49	—	— scar and areolar tissue only
9F	14	29	—	+ well differentiated node, 1.9 × 3 mm
9G	14	49	—	+ well-differentiated node, 3 mm diam.
1D	17	31	—	+ well-differentiated node, 2 × 3 mm
10A	17	60	—	area of experiment lost in preparation
10B	17	60	—	∓ scattered lympho-reticular islands
10D	17	60	—	∓ scattered lympho-reticular islands
10E	17	60	—	area of experiment lost in preparation
1C	26	19	—	+ poorly-differentiated node, 2 × 3.5 mm
6A	31	33	—	∓ scattered lympho-reticular islands
6B	31	33	—	∓ scattered lympho-reticular islands
6C	31	33	—	+ poorly-differentiated node, 1 mm diam.
6A-A	32	25	—	+ well-differentiated node, 1.7 × 2.3 mm
6A-C	32	25	+	+ well-differentiated node, 2.5 × 5 mm

31 rabbits.

* = hematoma developed after operation.

x = animal died before termination of experimental period.

minus (−) sign means that there is no indication of node regeneration, the sign $\mp$ that there is lympho-reticular tissue [2] but uncertainty as to the possibility of regeneration, the sign $\pm$ that this possibility is stronger, and the plus (+) sign, that there is no question of the presence of a regenerating lymph node.

As already indicated, this group furnished the basic experience and knowledge from which the later phases of the experiment could proceed more definitely.

Gross examinations of the specimens at autopsy revealed only 1 specimen which appeared to contain a node-like body. Microscopic examination of the sections showed, however, that, in all specimens except 1, extirpation is followed by an initiation of restorative activity in which the regenerating structure ranges from islands of simple lympho-reticular tissue to large lymphatic organs simulating the original nodes in complexity. Although the circumscribed lymphatic nodule probably represents the intermediate stage between the two extremes, the degrees of differentiation among the several specimens were so gradual as to contraindicate any attempts at finer classifications.

The 6 specimens containing well-differentiated nodes of regeneration are from the older animals of this group (14–32 days). Figure 5 illustrates a section through such a specimen in which a large regenerated node of about 2.5 × 5 mm lies deep to the scar of excision (*e*). The prominent cortex is occupied by several tiers of dense lymphatic nodules, the most peripheral ones forming distinct bulges on the capsular surface. The medulla consists of typical lymphatic cords and sinuses. Fibrous tissue surrounding the node forms a thin but definite capsule and blends with the scar at the superficial

[2] The writer finds it convenient to introduce and define the term "lympho-reticular" tissue, which consists chiefly of mesenchyme-like cells, with occasional lymphocytes, developing fat cells, macrophages, and fibroblasts interspersed among them. Microscopic islands of such tissue lie in the adipose-areolar tissue usually in close proximity to the plexus of reestablished lymphatics, especially in those specimens lacking a regenerated node or even in cases in which the latter is poorly differentiated.

pole. Trabeculae are scant but invade the parenchyma in a typical manner.

Afferent lymphatics which course from the scar empty into the marginal sinus at the superficial pole. A striking feature, however, is that a large share of the afferent stream continues uninterrupted past the node — as traced in serial sections and as indicated by arrows in figure 5 — to join the smaller efferent channel emerging from the deep pole of the node. The confluence of the 2 vessels at the latter point forms the main efferent trunk.

The lympho-reticular tissue, which appears to represent the aftermath of node extirpation in most of the specimens of this group and which was found even in the 2 animals with short post-operative periods of 5 and 9 days respectively, is depicted in figure 6. The cellular island, microscopic in size, lies below the scar in the adipose-areolar tissue near the course of the reestablished lymphatics (indicated by arrows in figure). Large, stellate-shaped reticular cells form a loose mesh which contain near its periphery, small and medium-sized lymphocytes. Mononuclear phagocytic cells with cytoplasmic inclusions and developing fat cells are also present. The lympho-reticular island lacks a capsule and its outer border merges insensibly with the surrounding adipose-areolar tisse. In some of the other specimens such islands were invaded by a rich plexus of small hemal capillaries.

B. Group II of young and near adult animals, with bilateral extirpation of the popliteal lymph node. This group represents a continuation of the study of the incidence in node regeneration among young and near-adult animals. Each age category, or subgroup, includes animals in a number sufficient to substantiate the experimental findings.

27- to 31-day rabbits (25 animals; post-operative period, 8–47 days). As shown in table 3-A, this age category consists of rabbits subjected to bilateral extirpation of the popliteal node at 1 month of age and sacrificed at the end of a post-operative period of 30 days. It should be mentioned that the first 9 animals (no. 12, A to K) were weaned after only 29 days.

TABLE 3-A

Summary of results. Group II — subgroup 1.

ANIMAL NO.	AGE, DAYS	POST-OP. PERIOD, DAYS	NODE REGENERATION	
			Gross	Microscopic
12A	27	18x	lt —	∓ scattered lympho-reticular islands
			rt +	+ poorly-differentiated node, 1.5 × 3.7 mm
12B	27	8x	lt —	∓ lympho-retic. islands in granulation tissue
			rt —	∓ lympho-retic. islands in granulation tissue
12C	30	31	lt —	∓ scattered lympho-reticular islands
			rt —	∓ scattered lympho-reticular islands
12D	30	31	lt —	+ well-differentiated node, 1.4 × 1.9 mm
			rt —	— scar and areolar tissue only
12E	30	31	lt —	∓ scattered lympho-reticular islands
			rt —	area of experiment lost in preparation
12F	30	31	lt —	area of experiment lost in preparation
			rt*—	∓ scattered lympho-reticular islands
12G	30	18x	lt —	∓ scattered lympho-reticular islands
			rt —	∓ scattered lympho-reticular islands
12H	30	22x	lt —	+ poorly-differentiated node, 1 × 1.7 mm
			rt —	± lymphatic nodule, 0.12 mm diam.
12I	30	47	lt —	∓ scattered lympho-reticular islands
			rt —	± lymphatic nodule, 0.5 mm diam.
12J	30	18x	lt —	∓ scattered lympho-reticular islands
			rt —	∓ scattered lympho-reticular islands
12K	30	23x	lt —	∓ scattered lympho-reticular islands
			rt —	— scar and areolar tissue only
14A	31	31	lt +	+ well-differentiated node, 3 × 5 mm
			rt —	± lymphatic nodule, 0.5 × 1.3 mm
14B	31	31	lt —	± lymphatic nodule, 0.2 × 0.3 mm
			rt —	+ poorly-differentiated node, 1 mm diam.
14C	31	31	lt —	+ well-differentiated node, 1.5 × 3 mm
			rt —	∓ scattered lympho-reticular islands
14D	31	31	lt +	+ well-differentiated node, 2 × 3.5 mm
			rt +	+ well-differentiated node, 1.2 × 2.5 mm
14E	31	31	lt —	+ poorly-differentiated node, 0.9 × 1.4 mm
			rt +	+ well-differentiated node, 3 mm diam.
14F	31	31	lt —	+ well-differentiated node, 1.3 × 1.9 mm
			rt +	+ well-differentiated node, 2.1 × 3.2 mm
14G	31	31	lt —	∓ scattered lympho-reticular islands
			rt +	+ well-differentiated node, 1.7 × 3.2 mm
14H	31	31	lt —	area of experiment lost in preparation
			rt —	area of experiment lost in preparation
15A	30	31	lt —	± lymphatic nodule, 0.1 × 0.3 mm
			rt —	∓ scattered lympho-reticular islands
15B	30	31	lt —	∓ scattered lympho-reticular islands
			rt +	+ well-differentiated node, 1.7 × 3 mm
15C	30	31	lt —	+ well-differentiated node, 2.3 mm diam.
			rt —	+ poorly-differentiated node, 0.6 mm diam.
15D	30	31	lt —	∓ scattered lympho-reticular islands
			rt —	+ well-differentiated node, 1 × 2 mm
15E	30	31	lt —	+ poorly-differentiated node, 1 mm diam.
			rt +	+ well-differentiated node, 3 × 5 mm
15F	30	31	lt —	± lymphatic nodule, 0.5 mm diam.
			rt —	∓ scattered lympho-reticular islands

25 rabbits.

* = hematoma developed after operation.

x = animal died before termination of experimental period.

(The usual time of weaning is between 45 and 50 days.) Six of these rabbits died prematurely from intercurrent infection following the surgical operation.

In the total of 50 extirpations, positive regeneration of the lymph node occurred in 25. This includes 8 bilateral and 9 unilateral regenerations. Although exhibiting the usual developmental variations, the majority here represent well-developed structures having the features of true lymph nodes. Their average size is well over 1 mm in minimum diameter, with the larger ones approximating the dimensions of the original popliteal nodes of 30-day animals.

A typical representative of what appears to be a nearly completed stage of regeneration of a popliteal lymph node shows a well-differentiated, normal cortex and medulla. A fibrous capsule envelops the entire organ and sends irregular and rather broad trabeculae between the cortical nodules into the medulla. Higher magnification discloses the typical features. Slightly beyond the middle of the node measuring here approximately 2×3.5 mm, it is traversed by a prominent axial shunt (fig. 7); although this passage along its course has communications with the medullary sinuses, the greater volume of the afferent lymph passes directly into the efferent trunk at the hilum. In the regenerated node of the opposite leg of the same animal the sinuses adjacent to the central shunt contain, for the first time, particles of the ink which was injected as a routine procedure into the leg and foot of all animals prior to sacrifice. Closer examination of the shunt in this case reveals, however, that its caliber is markedly reduced as the hilum is approached.

Figure 8 exhibits the field of regeneration in the several specimens of this age category in which the degree of nature of the restoration was not as advanced. A simple lymphatic nodule lies adjacent to the wall of one of the reestablished lymphatic vessels. Although microscopic in size, this nodule when compared with a typical lympho-reticular island (cf. fig. 6) possesses structural qualities characteristic of lymphatic tissue. The nodule shows a preponderance of small and

medium-sized lymphocytes which partially mask the reticular stroma. The compact mass is permeated by a rich hemal plexus which stems primarily from a thin-walled arteriole at its deep pole. Although the wide peripheral space which surrounds the nodule shows extensions corresponding in position to afferent and efferent lymphatic vessels, these are not united with the neighboring lymph stream.

Of the remaining 25 cases in this subgroup which contain neither regenerated nodes nor lymphatic nodules, 19 have scattered lympho-reticular islands. As usual these lie close to the reformed lymph vessels at the site of surgical intervention. Two specimens contain only plexuses of reestablished lymphatics which pass through the areolar tissue below the scar. Unfortunately in 4 of the other specimens the sections missed the area of experiment because of poor histological technique.

A feature, to which reference was made above and which is expressed most clearly in the present age group, needs to be described more fully now. In the normal rabbit the afferent lymphatic vessels arising in the subcutaneous tissue of the leg converge towards the superficial pole of the popliteal node. Actually, the entrances of these vessels into the marginal sinuses are distributed rather uniformly over the greater part of the perimeter of the node, the hilum being the only region devoid of the entering flow. To the regenerated node, on the other hand, the afferent lymphatics pass always in a tortuous manner through the overlying scar to reach its superficial pole. There they break up into many channels of irregular size which, after some of them empty directly into the perinodular sinuses at the superficial pole, pursue either 1 of 2 courses: (1) a large by-pass outside the node (diagram D, fig. 2) that continues deep as the main lymphatic trunk and receives the efferents from the deep pole of the node, and (2) a large central shunt that continues axially through the node towards its deep pole, giving collaterals to the sinuses located along its course. In this manner the central shunt decreases in relative caliber as the efferents at the deep or hilar pole are

approached. Since the pattern of the lymph flow just described cannot be displayed adequately in single histologic sections, a series of diagrammatic reconstructions is offered in figure 2.

45-day rabbits (12 animals, post-operative period, 30-32 days). This subgroup includes rabbits subjected to bilateral extirpation of the popliteal node at 45 days of age and sacrificed 1 month later. The incidence of reformed nodes, repre-

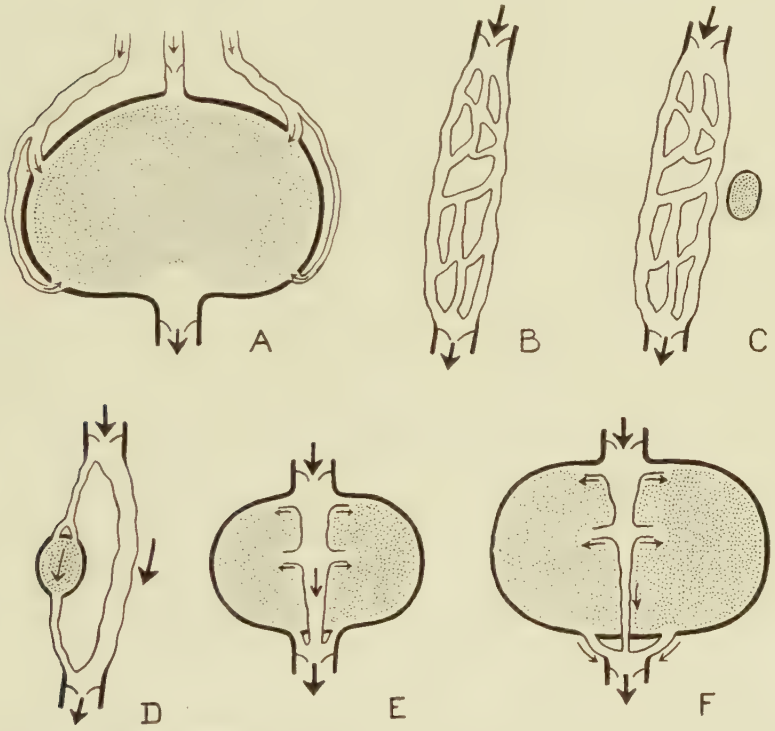

Fig. 2 Diagrams illustrating the types of lymphatic pattern in the original and the regenerated popliteal lymph nodes of rabbits. Arrows indicate direction and volume of flow based on the caliber of the lymph vessels. *A*. Normal arrangement of lymphatic vessels in original nodes. *B*. Reestablishment of severed lymphatics by formation of plexiform channels; no regeneration of lymphatic tissue. *C*. Regeneration of a simple lymphatic nodule adjacent to reestablished lymphatics. *D*. Diversion of part of lymph stream through a regenerated nodule or a poorly differentiated (regenerated) node. Most of afferent lymph channeled through a large by-pass. *E*. Well-differentiated node of regeneration with a prominent axial shunt. *F*. Completed lymph node of regeneration with a small axial shunt.

sented by 11 bilateral and 2 unilateral regenerations, is the highest encountered in the present study. More striking, however, is the large size of these nodes, associated with a highly advanced state of structural differentiation. But for a single specimen, they average well over 3 mm in their lesser

TABLE 3-B

Summary of results. Group II — subgroup 2.

ANIMAL NO.	AGE, DAYS	POST-OP. PERIOD, DAYS	NODE REGENERATION	
			Gross	Microscopic
13A	45	31	lt +	+ well-differentiated node, 3.5 mm diam.
			rt +	+ well-differentiated node, 6 mm diam.
13B	45	31	lt +	+ well-differentiated node, 2.9 mm diam.
			rt +	+ well-differentiated node, 3.6 mm diam.
13C	45	31	lt +	+ well-differentiated node, 4.8 × 5.7 mm
			rt +	+ well-differentiated node, 4.2 × 5.7 mm
13D	45	31	lt +	+ well-differentiated node, 2 × 3 mm
			rt +	+ well-differentiated node, 4.6 × 6.7 mm
13E	45	31	lt —	∓ scattered lympho-reticular islands
			rt +	+ well-differentiated node, 2.1 × 3.2 mm
13F	45	31	lt +	+ well-differentiated node, 4.5 mm diam.
			rt +	+ well-differentiated node, 3.2 × 5.3 mm
13G	45	31	lt +	+ well-differentiated node, 3.2 × 5.3 mm
			rt*+	+ well-differentiated node, 2 × 4 mm
13H	45	31	lt +	+ well-differentiated node, 4 mm diam.
			rt*—	+ poorly-differentiated node, 1 × 1.7 mm
13I	45	32	lt +	+ well-differentiated node, 2.1 × 3.8 mm
			rt —	∓ scattered lympho-reticular islands
13J	45	30	lt +	+ well-differentiated node, 2.5 × 4 mm
			rt —	+ well-differentiated node, 1.9 × 2.9 mm
13K	45	30	lt +	+ well-differentiated node, 5 mm diam.
			rt +	+ well-differentiated node, 5.6 × 6.3 mm
13L	45	30	lt +	+ well-differentiated node, 3.2 × 5 mm
			rt*+	+ well-differentiated node, 3.2 × 5.3 mm

12 rabbits.

* = hematoma developed after operation.

diameter. Moreover, in 15 specimens the sinuses of a node hold sufficient quantity of injected ink to be recognized macroscopically at the time of sacrifice (table 3-B).

Figure 9 depicts a central section from a typical regenerated specimen. Its size, being approximately 4.5 mm in diam-

eter, is comparable to the average dimensions of an original popliteal node of a 45-day animal. The morphologic features are essentially normal except for the presence of the axial shunt which seems to be constant for regenerated nodes that are well-differentiated. Although a considerable amount of injected ink is retained in the sinuses surrounding the central channels, some of the latter can be traced directly to the efferent trunk at the hilar pole. The fibrous trabeculae, passing through the parenchyma from the outer capsule, are much thickened around the collaterals of the central shunt.

The over-all picture of the nodes that retain injected ink and those free of it after injection is essentially similar, inclusive of the partial shunting of their afferent vessels. But in the former the caliber of the shunt is somewhat reduced at the hilar segment (compare E and F in fig. 2).

The exceptionally small regenerated node, referred to above, is approximately 1×1.7 mm and lies deep to the scar of excision. Its parenchyma shows the beginning of differentiation into a cortex and a medulla, but the stroma of the latter does not reveal the loose reticular network characteristic of the definitive stage. Judging from the arrangement of the lymphatic channels, only a small volume of the afferent lymph, streaming from the overlying scar, enters the node, while the remainder flows to one side of it via a large by-pass (D, fig. 2).

Finally, the 2 specimens, in which neither regenerated nodes nor lymphatic nodules are found, contain small lymphoreticular islands in the adipose tissue below the scar.

60-day rabbits (15 animals; post-operative period, 18–32 days). This subgroup consists of 2-month rabbits which underwent bilateral extirpation of the popliteal node and were sacrificed a month later. In the total of 30 excisions, positive regeneration of lymphatic tissue occurred in 19. This number, derived from 7 bilateral and 5 unilateral regenerations, yielded 15 specimens with features of true nodes, while 4 represented small, simple lymphatic nodules (table 3-C).

In contrast with the findings in the 45-day rabbits, the regenerated nodes in this subgroup are generally smaller, aver-

aging about 2 mm in its lesser diameter. Morphologically, too, they fail to equal the high degree of differentiation in the previous subgroup. Only 4 specimens show retention of ink in the nodal sinuses. One of these is a small, relatively undifferentiated lymphatic nodule, described below. The peculiar

TABLE 3-C

Summary of results. Group II — subgroup 3.

ANIMAL NO.	AGE, DAYS	POST-OP. PERIOD, DAYS	NODE REGENERATION	
			Gross	Microscopic
19A	60	32	lt —	∓ scattered lympho-reticular islands
			rt +	+ well-differentiated node, 3 × 4.2 mm
19B	60	32	lt +	+ well-differentiated node, 3.8 × 6.5 mm
			rt —	∓ scattered lympho-reticular islands
19C	60	32	lt +	+ well-differentiated node, 3 × 5 mm
			rt —	± lymphatic nodule with ink, 0.8 × 1.5 mm
19D	60	30	lt —	± lymphatic nodule, 0.5 mm diam.
			rt —	∓ scattered lympho-reticular islands
19E	60	30	lt —	+ well-differentiated node, 2 × 3 mm
			rt +	+ well-differentiated node, 1.7 × 3.5 mm
19F	60	18x	lt*—	+ poorly-differentiated node, 1 ×1.5 mm
			rt —	+ poorly-differentiated node, 0.8 × 2 mm
19G	60	30	lt —	+ poorly-differentiated node, 1 mm diam.
			rt —	+ well-differentiated node, 2 mm diam.
19H	60	30	lt*—	+ poorly-differentiated node, 0.7 × 1 mm
			rt —	— scar and areolar tissue only
19I	60	30	lt —	∓ lympho-reticular islands around abscess
			rt*—	∓ scattered lympho-reticular islands
19J	60	31	lt —	+ poorly-differentiated node, 1.5 mm diam.
			rt +	+ well-differentiated node, 2 × 3 mm
19K	60	31	lt —	∓ scattered lympho-reticular islands
			rt +	+ well-differentiated node, 3 × 4 mm
19L	60	31	lt —	∓ scattered lympho-reticular islands
			rt —	— scar and areolar tissue only
19M	60	31	lt —	± lymphatic nodule, 0.3 × 1 mm
			rt —	+ poorly-differentiated node, 1.5 mm diam.
19N	60	31	lt +	+ well-differentiated node, 2 × 4 mm
			rt —	± lymphatic nodule, 0.5 mm diam.
19O	60	31	lt —	— scar and areolar tissue only
			rt —	∓ scattered lympho-reticular islands

15 rabbits.

* = hematoma occurred after operation.

x = animal died before completion of experimental period.

disposal of the lymphatics, which so far has been found to be a constant feature of the regenerated nodes, is also encountered here.

Figure 10 illustrates a representative of the optimum stage of regeneration among the animals of the 60-day age group. This node, lying on the deep aspect of the scar tissue and measuring approximately 2 mm in diameter, possesses several large nodules at the superficial pole. The medulla is typical in the hilar region, but the greater more central part of it contains cells which, because of their abundant protoplasmic processes, seem comparatively primitive. The afferent lymphatics take their way through the scar and empty into large irregular vessels that, after giving off several small collaterals to the node, continue deeply to join the main efferent trunk. Several sections beyond the one photographed, the trunk collects the small efferent lymph vessels coming from the deep pole. The fibrous capsule of the node is thin, and the trabecular processes are relatively inconspicuous.

Figure 11 typifies the appearance of the 4 specimens in which regeneration did not progress beyond the stage of a lymphatic nodule. The nodule measures approximately 0.3×1 mm and lies near the path of the reestablished, plexiform lymphatics that descend from the scar to the large efferent trunk (visible at the left of the figure). When examined serially, most of the descending afferents are seen to proceed around the nodule with only few collaterals of capillary dimensions emptying into its superficial pole. Several small efferents, comparable in caliber to the afferent collaterals, emerge from the deep pole and join the main trunk. The nodule consists of a simple lymphatic mass without differentiation into cortex and medulla. The large clefts in it are artefacts produced by shrinkage. A thin, fibrous capsule only partially encircles the nodule, the exposed outer boundary of the latter merging with the perinodular tissue.

In 1 specimen the regenerated nodule shows retained particles of ink amongst the aggregation of lymphocytes near the

superficial pole. Its structural features, however, are similar to the one just described.

In 8 specimens in which neither regenerated nodes nor nodules exist, the popliteal tissue contains lympho-reticular islands. These lie in close proximity to the walls of the re-established lymphatics.

Finally, in 3 specimens both lymphatic and lympho-reticular tissues are absent, and the plexuses of reestablished lymphatics meander through areolar tissue.

90- to 96-day rabbits (15 animals; post-operative period, 31–102 days). Of these 3-month rabbits, subjected to bilateral extirpation of the popliteal lymph node, 10 had post-operative periods of 1 month, while 5 had between 45 and 102 days before reexamination of the operative site (table 3-D).

In the total of 30 extirpations, regeneration occurred bilaterally in 3 and unilaterally in 4 animals, giving a total of 10 newly-formed lymph nodes. These average approximately 2.5 mm in their lesser diameter, and 9 exhibit a high degree of structural restoration. In 5 of them the nodal sinuses hold ink particles from injections made before sacrifice. The arrangement of the afferent and intra-nodal lymphatics is similar to that observed in all nodes of regeneration. The tenth specimen represents a small, poorly-differentiated node about 1.2 mm in diameter. Serial sections, however, reveal that despite its structural simplicity this node is already connected with the neighboring main lymphatics by minute channels.

Although the specimen portrayed in figure 12 can be taken as an example of the well-differentiated nodes of regeneration in this age category, it was selected because of its history. It is approximately 4 mm in diameter and is from the left leg in an animal with bilateral regeneration. Despite the long period of restoration (99 days), the node is not significantly different structurally from nodes having a shorter post-operative period. Moreover, the pattern of lymphatic distribution, especially the prominent axial shunt, persists unaltered. The regenerated node of the right leg is slightly smaller, being

approximately 3 × 4.2 mm; its qualitative differentiation is similar.

As shown in table 3-D, 7 specimens contain large, solitary abscesses, each measuring about 2 cm in diameter, in the territory of lymph node removal. At the time of autopsy, an

TABLE 3-D

Summary of results. Group II — subgroup 4.

ANIMAL NO.	AGE, DAYS	POST-OP. PERIOD, DAYS	NODE REGENERATION	
			Gross	Microscopic
16C	90	31	lt —	∓ scattered lympho-reticular islands
			rt —	∓ lympho-reticular islands around abscess
16D	90	31	lt —	∓ lympho-reticular islands around abscess
			rt +	+ well-differentiated node, 3 × 4 mm
16E	90	31	lt +	+ well-differentiated node, 2 × 4 mm
			rt —	— scar and areolar tissue only,
16F	90	31	lt —	+ well-differentiated node, 2 × 2.7 mm
			rt —	+ well-differentiated node, 1.5 × 2 mm
16G	90	31	lt —	∓ scattered lympho-reticular islands
			rt —	∓ lympho-reticular islands around abscess
17A	95	31	lt —	∓ lympho-reticular islands around abscess
			rt —	— scar and areolar tissue only
17B	95	99	lt +	+ well-differentiated node, 4 mm diam.
			rt +	+ well-differentiated node, 3 × 4.2 mm
17C	95	102	lt —	area of experiment lost in preparation
			rt —	— scar and areolar tissue only
17D	95	31	lt —	— scar and areolar tissue only
			rt +	+ well-differentiated node, 3 mm diam.
17E	95	31	lt —	+ poorly-differentiated node, 1.2 mm diam.
			rt —	∓ lympho-reticular islands around abscess
17F	95	31	lt +	+ well-differentiated node, 2.5 × 4 mm
			rt +	+ well-differentiated node, 3.2 × 4 mm
17G	96	47	lt —	∓ scattered lympho-reticular islands
			rt —	∓ scattered lympho-reticular islands
17H	96	30	lt —	∓ lympho-reticular islands around abscess
			rt —	— scar and areolar tissue only
17I	96	45	lt —	∓ scattered lympho-reticular islands
			rt —	∓ scattered lympho-reticular islands
17J	96	102	lt*—	— scar and areolar tissue only
			rt*—	∓ lympho-reticular islands around abscess

15 rabbits.

* = hematoma developed after operation.

abscess was easily palpable as a firm, capsulated sphere in the popliteal space, which upon incision contained a creamy, semi-solid pus. Upon microscopic examination the abscess cavity was seen to be filled with degenerated blood cells in which polymorphonuclear leucocytes were the predominant type. The wall of the abscess has 2 fairly distinct layers: an inner one, several millimeters thick, composed of relatively avascular fibrous tissue that lies next to the necrotic content, and an outer vascular one consisting of a loose granulation tissue (fig. 13). In the outer wall of the abscesses characteristic lympho-reticular islands, which contain lymphocytes predominantly, are found near the small hemal capillaries.

The course of the main lymph vessels in the specimens complicated by infection could not be traced satisfactorily in each case. But in 2 specimens the reestablished lymphatics from the overlying scar encircle the wall of the abscess to reach the depths of the popliteal space. As to the occurrence of the infections, the writer is unable to offer any definite explanation, for the standards of the surgical technique had not been changed in any way.

In 6 specimens in which neither regenerated lymph nodes nor lymphatic nodules were evident, the areolar tissue occupying the site of extirpation possesses numerous patches of lympho-reticular tissue. These patches, some measuring as much as several millimeters across, lie in the areolar tissue deep to the scar where they maintain their usual close spatial relationship to the plexus of reestablished lymphatics.

In another 6 specimens the area from which the node was excised contains only restored lymphatics. Finally in 1 specimen the area of regeneration was lost because of technical errors in sectioning.

120- to 153-day rabbits (16 animals; post-operative period, 30–32 days). The animals comprising this subgroup were subjected to bilateral extirpation of the popliteal node at a period in their life cycle intermediate between youth and full adulthood. The post-operative period was 1 month (table 3-E).

From 4 bilateral and 3 unilateral regenerations, 7 of the 11 specimens exhibited features of newly-formed lymph nodes, including a single specimen with retained ink, while in the other 4 restoration did not progress beyond the nodular stage.

TABLE 3-E

Summary of results. Group II — subgroup 5.

ANIMAL NO.	AGE, DAYS	POST-OP. PERIOD, DAYS	NODE REGENERATION	
			Gross	Microscopic
16A	120	31	lt +	+ well-differentiated node, 2 mm diam.
			rt —	+ poorly-differentiated node, 1 mm diam.
16B	120	31	lt —	∓ lympho-reticular islands around abscess
			rt —	∓ lympho-reticular islands around abscess
21A	150	31	lt —	+ well-differentiated node, 2 × 3 mm
			rt —	— abscess only
21B	150	31	lt —	± lymphatic nodule, 0.7 × 1.5 mm
			rt —	∓ scattered lympho-reticular islands
21C	150	31	lt —	+ poorly-differentiated node, 1.7 × 3.8 mm
			rt —	∓ scattered lympho-reticular islands
21D	150	30	lt —	± lymphatic nodule, 0.2 × 0.3 mm
			rt —	∓ lymphatic nodule, 0.9 × 1.3 mm near abscess
21E	150	30	lt —	+ well differentiated node, 2 × 4 mm
			rt —	± lymphatic nodule, 0.5 mm diam. near abscess
21F	150	30	lt*—	∓ scattered lympho-reticular islands
			rt —	∓ scattered lympho-reticular islands
21G	150	30	lt —	∓ lympho-reticular islands around abscess
			rt —	∓ scattered lympho-reticular islands
21H	150	31	lt —	— scar and areolar tissue only
			rt*—	∓ scattered lympho-reticular islands
21I	150	31	lt +	+ well-differentiated node, 2.9 mm diam.
			rt +	+ well-differentiated node, 2.2 × 2.7 mm
21J	150	31	lt —	∓ scattered lympho-reticular islands
			rt —	∓ lympho-reticular islands around abscess
21K	150	31	lt —	∓ scattered lympho-reticular islands
			rt —	∓ scattered lympho-reticular islands
21M	150	31	lt*—	∓ scattered lympho-reticular islands
			rt*—	∓ lympho-reticular islands around abscess
21N	153	32	lt —	— scar and areolar tissue only
			rt —	∓ lympho-reticular islands around abscess
21O	153	32	lt —	∓ scattered lympho-reticular islands
			rt*—	∓ scattered lympho-reticular islands

16 rabbits.

* = hematoma developed after operation.

Both types possess the respective structural features characteristic of neoformation. But 2 of the 4 specimens containing the lymphatic nodules are each complicated by a large abscess, that occupies the center of the area submitted to experimentation. The regenerated nodule lies in the path of the displaced main lymphatic which, in its inward course from the scar, skirts the wall of the abscess (fig. 14).

The cases in which the experimental area contains only scattered lympho-reticular islands and reestablished lymphatics reach their highest relative incidence in this subgroup (120- to 153-day animals), as evidenced by 18 of the 32 specimens. In 6 of them abscesses were again present.

Three specimens, showing no lympho-reticular activity, have reestablished lymphatics passing through areolar tissue below the scar of excision. One of these possesses a central abscess.

C. Group III of adult and senile animals, with unilateral and bilateral extirpation of the popliteal lymph node. The question as to whether regeneration of lymph nodes occurs in adult and old-age rabbits was subjected to experimental test in this group. Little or nothing is known about this problem in senile animals. The procurement and maintenance of normal rabbits which have reached senility is, of course, fraught with many difficulties, and the writer was fortunate, indeed, in having been able to experiment on 6 animals with certified ages ranging from $2\frac{1}{2}$ to $3\frac{1}{2}$ years. According to the literature dealing with the possibility of regeneration of the popliteal node, adult animals had been studied quite extensively, although authors did not state specifically how old the "adults" were. In the present investigation, rabbits from 8–15 months were considered to be definitely adult, yet far from being senile.

8- to 15-month rabbits (10 animals; post-operative period, 63–330 days). This subgroup includes full-grown rabbits in which 7 bilateral and 3 unilateral extirpations of the popliteal lymph node were performed. The post-operative period, being

between 2 and 11 months (table 4), was of much longer duration than for previous groups.

From the total of 17 extirpations, only 1 case of positive regeneration occurred, this being in the left leg of an animal 10 months old at the time of operation and sacrificed after a post-operative period of 217 days. Although the node was

TABLE 4

Summary of results. Group III — adult and senile rabbits.

ANIMAL NO.	APPROX. AGE, DAYS	POST-OP. PERIOD, DAYS	NODE REGENERATION	
			Gross	Microscopic
R1b	9 mo.	202	rt —	— scar and areolar tissue only
S2	12 mo.	215	lt —	— scar and areolar tissue only
			rt —	— scar and areolar tissue only
S3	12 mo.	215	lt —	— scar and areolar tissue only
			rt —	∓ small lympho-reticular island
S5	9 mo.	68	rt —	— scar and areolar tissue only
S6	8 mo.	84	lt —	— scar and areolar tissue only
			rt —	— scar and areolar tissue only
S7	10 mo.	217	lt —	+ poorly-differentiated node, 2 × 2.5 mm
	8 mo.	87	rt —	— scar and areolar tissue only
S8	9 mo.	210	rt —	— scar and areolar tissue only
S9	15 mo.	106	lt —	∓ small lympho-reticular island
	12 mo.	208	rt —	— scar and areolar tissue only
S10	15 mo.	106	lt —	— scar and areolar tissue only
			rt —	— scar and areolar tissue only
S11	10 mo.	330	lt —	— scar and areolar tissue only
			rt —	— scar and areolar tissue only
OA6	3 yrs.	37	lt —	— scar and areolar tissue only
		70	rt —	— scar and areolar tissue only
OA9	3½ yrs.	91	lt —	— scar and areolar tissue only
			rt —	+ poorly-differentiated node, 1.5 × 2.2 mm
OA10	2½ yrs.	31	lt —	+ poorly-differentiated node, 2 mm diam.
		38	rt +	— residual node, 5 × 8 mm
OA11	3½ yrs.	73	lt —	+ poorly-differentiated node, 0.8 mm diam. deep to abscess
			rt —	— scar and areolar tissue only
OA12	3½ yrs.	46	lt —	— residual node, 1.8 × 2.1 mm
			rt —	∓ lympho-reticular islands around abscess
OA13	3½ yrs.	46	lt —	— scar and areolar tissue only
			rt —	± 2 lymphatic nodules, 0.4 × 0.6 and 0.8 × 1 mm

16 rabbits.

approximately 2×2.5 mm in size, it was not recognized macroscopically at autopsy. As shown in the section illustrated in figure 15, it lies on the deep aspect of the scar of excision and presents features typical for nodes of regeneration. The lack of a distinct cortex and medulla is more apparent than real because of the congestion of the sinuses with large numbers of free leucocytes, which include many eosinophiles. A thin fibrous capsule encloses the node completely, but the internal trabeculae are scant. Most of the afferent lymphatics from the overlying scar open directly into the subcapsular sinuses (filled with injected ink), while the others by-pass the node to join the main efferent trunk below the deep pole.

The specimen from the right leg of the same animal (S7, see table 4) does not show any signs of regenerative activity 3 months after the excision of its node.

In the remaining 15 specimens, 2 have small amounts of characteristic lympho-reticular tissue in the area of node excision, while in the other 13 cases such areas contain only adipose tissue through which pass reestablished lymphatics and blood vessels.

$2\frac{1}{2}$- to $3\frac{1}{2}$-year rabbits (6 animals; post-operative period, 31–91 days). The senile rabbits were subjected to bilateral removal of the popliteal lymph node and sacrificed after a post-operative period ranging from 1 to 3 months (table 4).

Three of the 12 specimens contain structures which exhibit a sufficient degree of restoration to be classified as poorly-differentiated nodes. In another specimen, 2 small, regenerated lymphatic nodules, one below the other, lie deep to the scar of excision in close proximity to the plexus of restored lymphatics.

Unorganized lympho-reticular tissue occurs only in 1 specimen, which was complicated by a large central abscess. In 3 specimens even this tissue is absent, the site of node extirpation being occupied by areolar tissue and plexiform channels of the reestablished lymphatics.

Finally in 2 specimens there is a structure which is unique in the present investigation. The first one measuring approximately 5×8 mm, occupies the site of excision and, on casual observation of a single central section, may give the impression of a large lymph node of regeneration (fig. 16). Yet, it possesses several significant peculiarities in structure that are found neither in original nor in regenerated nodes. Scattered throughout it are numerous vesicles of various sizes partially filled with cellular debris. In the center of the node, fibrous tissue forms a solid core in which are embedded occasional clumps of isolated lymphocytes. The cortical portion fails to show the normal features, and the lymphocytes there form a diffuse lymphatic mass. The peripheral sinus is abnormally wide and contains islands of dark-staining cells, mostly detached mononuclear phagocytes (fig. 17). The capsule around the node is doubled, for in addition to the inner covering, the perinodal areolar tissue has condensed into a second distinct layer which completely envelops the body. Except for few blood capillaries which penetrate the thick capsule, the node is wholly devoid of the larger afferent and efferent lymph and blood vessels. The lymphatics descending from the scar (segments of it appear in fig. 16), but failing to enter the node, pass to one side of it in order to reach the depths of the popliteal space.

Although the node in the second specimen is smaller in size, its structural peculiarities are almost identical with those of the one just described. The trabeculae of the cortex, however, show more diffuse fibrosis and the cortical sinuses contain multinucleated giant cells in addition to the free phagocytes (fig. 18).

These 2 lymph nodes present histologic features which point to their passive survival rather than to an active functional life after restoration. Diffuse fibrosis of the medulla in 1 node and giant cells in the cortical sinus of the other may even indicate a pathologic state leading towards degeneration. Because these 2 nodes are devoid of the afferent and efferent lymph vessels as well as the other characteristics typical for

all regenerated nodes, the writer suspects that they represent proliferations from residual portions of the original lymph nodes which it seems were not totally extirpated. This suspicion is supported by the observation that, when the respective serial sections of the original nodes were followed through, a small fraction of the cortex was found missing in each.

D. Mode of regeneration of the popliteal lymph node. Group IV (table 5) includes 22 young animals, 1½ months of age, which were subjected to bilateral extirpation of the popliteal node, and then sacrificed after a post-operative period ranging from 7 to 28 days; most of the cases fell between the beginning and the end of the third week, which was anticipated as the "critical period" of regeneration.

An investigation that demands an examination of a progression of developmental events — in this case, regenerative stages — based on fixed material, is subject to a number of conditions or limitations: (1) the sequence of stages must of necessity be derived from different specimens; (2) even though the post-operative periods for different animals be properly spaced, the factor of biological variation introduces uncertainties in the sequence; (3) successful regeneration cannot be expected in every specimen; (4) a greater number of specimens must be examined in order to set up a sequence of graded stages.

As the reader compares the photomicrographs (figs. 20–23) portraying the advancing stages of regeneration of the node, he must bear in mind the decreasing magnifications employed (and stated) in going from the earliest and smallest to the latest and largest stages; it is obvious why this should be so, but it is also unfortunate since it obscures the comparative size relations.

Table 5 summarizes the experimental history and results for each specimen within the arranged intervals, whereas in the succeeding descriptions only those specimens are considered which were selected as representative or typical stages in the progress of regeneration.

TABLE 5

Summary on the mode of regeneration of the popliteal lymph node.

POST-OPERATIVE PERIOD	RABBIT NO.	OBSERVATIONS
7 days	18C	
	lt, rt	Distended afferent lymphatics empty into cavity of extirpation; latter drains into remnant of original efferent trunk via plexiform channels. Beginning of lympho-reticular foci in granulation tissue. (Slight edema of leg and foot.)
14 days	18B	
	lt, rt	Reduced cavity of extirpation. Lympho-reticular islands more discrete and concentrated near efferent and afferent channels. Disappearance of distention of afferent lymphatics. (No edema of leg and foot.)
	20C	
	lt, rt	No cavity of extirpation. Small, vascularized lymphatic island along reestablished lymphatics.
16 days	20A	
	lt	(Small residual node.)
	rt	Scattered lympho-reticular islands along reestablished lymphatics.
	20H	
	lt, rt	Small lymphatic nodule in crotch of large reestablished lymphatic; nodule possesses small "afferent" and "efferent" lymphatics not connected to lymph stream.
18 days	18F	
	lt	5 lymphatic nodules clustered around reestablished lymphatics, the largest of which taps part of the lymph stream and shows the beginning of cortex and medulla.
	rt	Scattered lympho-reticular islands along reestablished lymphatics.
	20B	
	lt, rt	Scattered lympho-reticular islands along reestablished lymphatics.
	20I	
	lt, rt	Scattered lympho-reticular islands along reestablished lymphatics.
19 days	18D	
	lt, rt	Large lympho-reticular islands containing circumscribed condensation of lymphocytes.
	20L	Animal died before termination of experiment. Specimens discarded.
20 days	20C	
	lt, rt	Scattered lympho-reticular islands along reestablished lymphatics.

TABLE 5 (*continued*)

POST-OPERATIVE PERIOD	RABBIT NO.	OBSERVATIONS
20 days	20J	
	lt, rt	Small lymphatic nodule surrounded by tissue spaces.
21 days	18A	
	lt, rt	Scattered lympho-reticular islands along reestablished lymphatics.
	18E	
	lt	Well-differentiated node, 3.2 × 4.2 mm.
	rt	Well-differentiated node, 2.8 mm diam.
	20D	
	lt	Well-differentiated node, 2.5 × 3.6 mm.
	rt	Well-differentiated node, 1.2 mm diam.
	20K	
	lt, rt	Scattered lymphatic masses along reestablished lymphatics.
22 days	20E	
	lt	Poorly-differentiated node, 0.6 × 0.8 mm. Scattered lympho-reticular islands along reestablished lymphatics.
24 days	18G	
	lt	Area of experimentation missing from sections — poor preparation.
	rt	Well-differentiated node, 3.2 × 4.2 mm.
	18H	
	lt	Well-differentiated node, 3 × 4 mm.
	rt	Well-differentiated node, 4.2 × 5.3 mm.
	20F	
	lt	Poorly-differentiated node, 0.7 × 1.3 mm.
	rt	Scattered lympho-reticular islands along reestablished lymphatics.
28 days	18I	
	lt	Area of experimentation missing from sections — poor preparation.
	rt	Well-differentiated node, 2.3 mm diam.
	18J	
	lt	Scattered lymphatic masses along reestablished lymphatics.
	rt	Poorly-differentiated node, 1 × 1.2 mm.

7-day post-operative period. In the specimen taken, a cavity 2.5 mm in diameter and surrounded by fibrous scar tissue occupies the approximate site of extirpation (fig. 19). In the section, stained with hematoxylin and eosin, a pink-colored coagulum containing degenerated lymphocytes partially fills the lumen. The afferent lymphatics terminate above the scar as a plexus of distended channels. Some of these end blindly while a few proceed tortuously to open into the cavity. From the floor of the latter a large irregular channel continues deep towards the occluded stump of the nodal artery and there is confluent with the remnant of the efferent lymphatic trunk. Thus 1 week after the excision of the node there already has been established at least a provisional passage between the original afferent and efferent lymph channels. Typical lympho-reticular islands (*l.r.*) exist both between the plexus of afferent lymphatics and around the newly-formed junction with the efferent lymph trunk.

14-day post-operative period. A cavity, doubtlessly equivalent to the one pointed out in the previous stage, has become reduced and incorporated as a segment in the reestablished lymphatic pathway that descends from the scar and joins the stump of the main efferent trunk. Smaller collaterals which lie more peripherally provide additional communications with that trunk. Because of this better confluence there is no distended plexus in the afferent territory as was seen in the preceding specimen.

Lympho-reticular islands found at this stage contain a greater number of lymphocytes and appear more compact. Moreover, the islands, especially the larger ones which previously had a helter-skelter distribution, are now restricted to the immediate vicinity of the reformed junction between the afferent and efferent lymphatic channels deep to the scar tissue (fig. 20).

16-day post-operative period. The representative specimen has a body in which the stage of differentiation is intermediate between that of a simple lymphatic nodule and that of a young lymph node of regeneration (fig. 21). The body is situated

deep to the scar of excision between the branches of the re-established lymphatics (*r.l.*). Its upper or superficial portion consists of characteristic lympho-reticular tissue and contains small discrete spaces near its margin. At the deep pole, however, the tissue is more compact, being filled with lymphocytes, and is demarcated from the surrounding areolar tissue by a large cleft (*c*). From the latter a prominent hollow bayou projects deeply towards the lymph channels, but is still not in continuity with them.

18- to 20-day post-operative period. As exhibited in figure 22, there is definite advance in differentiation from the preceding stage, resulting in further complexity of the lymphatic tissue, as well as in the arrangement of the lymphatics. The specimen shows 5 regenerated lymphatic nodules of varying size and structure which are clustered around the reestablished lymph stream (*rnd. 1, rnd 2, rnd 3, rnd 4, rnd 5*). These nodules are connected to one another by strands of the same tissue. Nodule *rnd 1* not only is the largest but is the best differentiated. It possesses 1 large and several small lymphopoietic centers separated by a diffuse medulla. At the superficial border the medullary sinuses receive some of the lymphatics in the scar, while at the deep pole of the nodule they communicate with the main lymph trunk via short efferent channels. A moderately thick layer of fibrous tissue forms a capsule of the nodule. The smaller nodules *rnd 2, rnd 3* and *rnd 4* are all comparable in regard to size and development. Structurally, they are true nodules in the sense that they consist of compact and well-circumscribed lymphatic tissue. Collaterals from the main lymph trunk form a minute plexus around the periphery of each nodule. Finally, nodule *rnd 5* seems to be completely cut off from the lymph stream. It is the least advanced of the 5 bodies in differentiation, for it is still unorganized lympho-reticular tissue, lacking a definite capsule and blending directly with the areolar tissue.

21- to 22-day post-operative period. Apparently regeneration is about completed 3 weeks after node excision, for the specimen obtained at this time shows a well-differentiated

lymph node (fig. 23). The morphologic features are identical with those of the regenerated nodes encountered in Groups I and II.

24- to 28-day post-operative period. Despite a post-operative period longer by several days, the regenerated nodes at about 4 weeks differ little from those of the 3-week stage; in other words, they appear to have attained their limit of differentiation.

IV. DISCUSSION AND CONCLUSIONS

As was stated in the introduction, the questions pertaining to lymph node regeneration still awaited decisive answers, despite the many researches that have dealt with the problem in the past.

Though an adequate amount of material is one of the important requisites of sound experimental work, many of the published reports on lymph node regeneration overlooked this condition. Conclusions based upon insufficient material are often conflicting and at best provisional. Especially in the type of experimentation in which the technical dexterity of the investigator influences greatly the validity of the results, the factor of the number of experiments cannot be overemphasized. The removal of the lymph node, say in 10 or 20 animals is but a preliminary step, giving the investigator merely a basis of procedure. This point is well borne out by the results obtained from the writer's first group of experimental animals. Although the number of 31 rabbits (Group I) looks impressive, the observations on them are not definitely conclusive, even if suggestive of the significance of age as a factor in lymph node regeneration.

That the potentiality for regeneration is greater during the growth phase of an organism is a fact in biology. Yet this principle has been almost totally disregarded by those who have attacked the problem of reformation of lymph nodes. Evidently Meyer ('06) and Auché ('30) were cognizant of this point, for in their discussions both concede that the possibility of lymph node regeneration was greater in young, growing animals. They failed, however, to subject their

concession to experimentation. By far most of the 22 papers by other investigators represent reports on adult material, and in the few instances in which younger animals were used their exact ages were not given. That the regeneration of the lymph node is limited almost to young and near-adult rabbits has been demonstrated in the present investigation. In a total of 166 specimens, derived from bilateral extirpations in 83 rabbits which ranged in age from 1 to 5 months (Group II), regeneration of a new popliteal lymph node occurred in about 50%. The incidence becomes even more significant when this figure is broken down into specific age categories, as illustrated in the following: in 1-month-old rabbits the incidence is 50%, in 1½-month-old, 90%, in 2-month-old, 60%, in 3-month-old, 33%, in 4- to 5-month-old, 28%. Interesting, too, is the fact that in the 1½-month-old rabbits not only the incidence is at a maximum but the degree of structural restoration is also at a peak, as evidenced by their large, well-differentiated nodes of regeneration. The literature fails to reveal a single publication that gives figures of incidence of regeneration with which the present findings can be compared.

The writer's suspicion, that the incidence of lymph node regeneration may be associated with the normal growth cycle of the original lymphatic tissue of the animal, found substantiation in the comprehensive study of Hellmann ('13) on the post-natal weight changes of the lymphatic organs in the rabbit. His data on the growth of the popliteal lymph node in the normal animal showed a rapid increase in weight beginning at the first month and extending up to the fifth. Thereafter the curve plateaued and terminated in a gradual involutionary decline. It is seen that the age period in which regeneration of lymph nodes occurs falls within the rapidly ascending phase of Hellmann's growth curve. However, the age of optimum regeneration (1½ months) has no counterpart in that curve. In a search for information on the period of progressive body growth of the rabbit, the writer was fortunate in obtaining accurate, unpublished data through personal communication with Mr. George Templeton, Director of the Rabbit Experi-

ment Station of the United States Department of Agriculture. His results on the weight curves of young normal rabbits demonstrate the maximum rate of growth takes place from the twenty-eighth to the sixty-eighth day, an interval which coincides with the age categories having the 3 highest incidences of lymph node regeneration.

With respect to regeneration in the mature animals (Group III), the incidence was 1 out of 17, or 5% in adults, and 3 out of 12, or 25% in senile rabbits. The resurgence of regenerative potency in old age cannot be explained satisfactorily. More extensive studies on a larger number of senile rabbits may clarify this unexpected finding. Perhaps the writer's incidental observation that the lymph node and *its perinodal tissue* retain their capacity for further growth and differentiation even in senility, as evidenced by experimental activation (see page 450), may shed some light. In the senile specimens which possessed regenerated nodes, the necessary stimulus for their initiation could possibly have originated from the foci of superficial infections that are almost a common occurrence in old rabbits, at any rate in those maintained throughout their life in cages. Related studies by other investigators on senile rabbits could not be found. Publications of experiments on adult rabbits indicate that, except for the results reported by several Japanese workers, the popliteal node does not regenerate at this age. The validity of the conflicting reports will be discussed subsequently.

Although the obvious advantages of selecting an experimental area which contained but a solitary node were pointed out as early as 1886 by Bacialli, other investigators failed to heed such considerations. As a matter of fact in several instances even the least accessible visceral nodes have been the subject of experimentation in the study of lymph node regeneration (cf. Meyer, '06; Papillian and Russu, '31). In regions such as the groin, the axilla, or the neck, where lymph nodes occur in groups of variable numbers and dimensions, the possibility of incomplete removal becomes greater. Here, radical extirpation could be considered but this would intro-

duce factors which would completely vitiate the purpose of the investigation. According to the extensive studies by Rouvière and Valette ('37), supernumerary popliteal nodes in the rabbit occur too rarely to be of significance. The advantageous choice of the popliteal space as the locale of experimentation is proven in the present investigation, for in the 152 rabbits used, the writer failed to see a single supernumerary lymph node. A bilobed structure was encountered occasionally which gave an initial impression of multiplicity on macroscopic observation, but histologic examination of serial sections revealed its true nature.

The high degree of surgical accuracy required in the experimental removal of the lymph node cannot be overemphasized. This factor alone probably is responsible for much of the conflict of opinions, and it is unfortunate indeed that previous investigators have given it inadequate attention. A satisfactory operation in total ablation involves the removal of the *entire lymph node* with the minimum of injury to the residual perinodal tissue. For if a portion of the node is missed, be it even the smallest bit, the latter would permit, at least on theoretical grounds, a hyperplasia in lieu of true neoformation. On the other hand, if a radical removal, including all of the surrounding perinodal tissue, be substituted, then this will eliminate the possible source for node regeneration. In this respect the positive results which a few investigators obtained by the "complete shelling-out" of the node parenchyma alone, or the negative results, namely failure of regeneration following total removal of the popliteal contents, are in either case to be accepted with caution. Although not falling within the scope of this discussion, the dispute as to whether a resected (that is, partially removed) lymph node will regenerate is not entirely settled. Probably such factors as availability of blood supply, infections, or the extent of tissue trauma influence the fate of the residual portion. That there exist certain histologic differences between true nodes of regeneration and remnants of incomplete extirpation have been demonstrated by 2 specimens which the writer obtained

from senile animals. Although these specimens possessed a superficial resemblance to regenerated nodes, they had undergone degenerative changes after an initial period of proliferation and enlargement. Whether they would have recovered again if the time had been extended — these specimens represented post-operative periods of 38 and 46 days, respectively — is doubtful.

Analysis of the literature bearing on the subject of lymph node regeneration reveals that in some of the investigations the conclusions were based mostly on results ascertained from macroscopic observations. As expected, practically all of these reported the absence of regenerative activity. The inadequacy of such observations is proven by the writer's experience that histologic examination confirmed the reliability of gross examinations only if the regenerated structures were of sufficient magnitude, or their contrasts were enhanced by coloring material, such as India ink. The latter procedure, however, is not an absolute indicator because of numerous variable and uncontrollable factors. As one should expect, the retention of ink particles in regenerated nodes is dependent to a large degree on the structural relationship between the node and its afferent lymphatic vessels, yet in some cases nodes were clear of such injection, notwithstanding the fact that these were structurally similar to the nodes showing abundant retention.

Since the main emphasis in the past has been placed on the incidence of node regeneration, the equally important aspect of the *mode* of neoformation has received only secondary attention. Ideas concerning the ultimate source from which the new node arises are numerous, although it must be said that many of these are speculations unsupported by experimental evidence. The claim by Bayer (1885) that neoformation of lymph nodes in the adult animal resulted from the direct transformation of adipose tissue was upheld for a brief time by a few of his immediate successors. Although Delius (1888) was the first to believe in the "fixed tissue elements" as the responsible precursor, Aucké ('30) was the first to demon-

strate experimentally that lymphatic tissue regenerates from undifferentiated cells isolated in the perinodal areolar tissue. The lymphocyte has not been considered as significant in the initiation of the regenerative process.

By studying closely-spaced stages of differentiation in young rabbits (Group IV), the writer was able to follow the developmental sequence involved in neoformation of the popliteal lymph node. The general picture of this progress was found to agree with that of the morphogenesis of the original node in prenatal life. But the possession of a "lymphatic shunt," described by the writer, is a pecularity specific for all completed nodes of regeneration. The intermediate stages leading from lympho-reticular islands to the characteristic lymph node of regeneration were so fully represented in the writer's material as to permit reconstruction of the entire mode of neoformation in a clear and conclusive manner. In the only other publication which makes an attempt to present the steps of this process, Auché ('30) succeeded in observing only the initial and the terminal steps. Evidently he did not have enough specimens to study the critical stages, nor did he resort to the use of complete serial sections in his work, for he admits that he was unable to find a single preparation which displayed the relations of the lymph vessels to the regenerated node. Needless to say, the discrepancies between Auché and the writer's findings emphasize again the importance of at least 2 of the cardinal principles of the kind of investigative work embodied here, namely, the adequacy of experimental material and the completeness with which this material is prepared for study.

Finally, a word may be said concerning the stimulus which initiates the restorative process after removal of the node. Although Bayer (1885) believed that lymph stasis was the necessary factor, his view was vigorously opposed by later workers. These men were of the opinion that irritation was essential for induction of regeneration and hence applied sterile foreign material, such as graphite, carmine, gelatine, sponge at the site of node excision. According to Auché ('30),

regeneration occurs only where a node was present previously, or in his own words, "in sites of node suppression." Rouvière and Vallete ('37) believed that regeneration of a new node was dependent upon the quantity of lymph flowing to it. While these statements may be accepted as corollaries to experimental observations, they are nevertheless tinged with considerable abstract teleology. Perhaps a more satisfactory explanation may eventually come from the newer science of chemical morphogenesis — with "organizers" as the inductive factors.

V. SUMMARY

In this experimental study on lymph node regeneration the popliteal node was removed surgically in 152 rabbits, ranging in age from 3 days to 3½ years. Unilateral and bilateral excisions gave a total of 270 specimens which furnished the basis for observations.

Neoformation of lymph nodes took place at the site of excision but this neoformation was dependent on the age of the animal. Although it occurred in about 50% of young and near-adult animals, the optimum age range both as to incidence and to quality of structural differentiation was approximately at 45–60 days.

The inclusion of specimens which contained only unorganized lympho-reticular islands shows that the potentiality for regeneration remains at a much higher level (approximately 80–90%) in the young and near-adult animals.

Reformation of lymph nodes in adult animals (8–15 months) was negligible. Its resurgence, however, in senile rabbits (2 to 3½ years) was inconclusive because of the limited number of specimens available for study.

The sequence of events in reformation generally simulate the normal morphogenesis of the original lymph node. The anlage begins as a small island of mesenchymal cells (lympho-reticular tissue) in the vicinity of the reestablished lymphatics, and differentiates into a lymphatic nodule. The conversion of the latter into a true node requires about 3 to 4 weeks. The

completed nodes of regeneration possess a lymphatic by-pass or shunt, a feature which was found to be specific for them.

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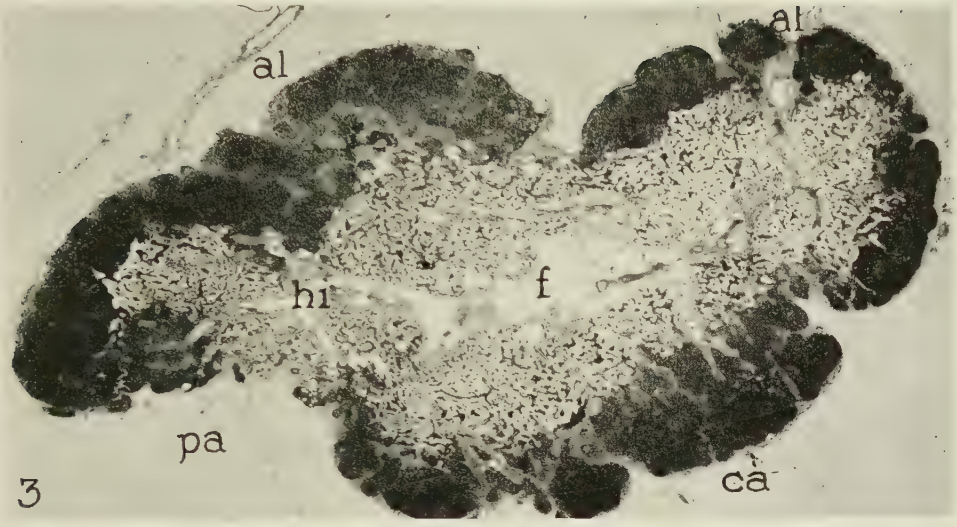
PLATE 1

EXPLANATION OF FIGURES

Figures 3–23 inclusive, are unretouched photomicrographs.

3 Section through the right popliteal lymph node of a 3-year-old rabbit. × 10.5. *f*, adipose tissue extending from hilum; *hi*, into medulla; *ca*, capsule; *al*, afferent lymphatics; *pa*, perinodal areolar tissue.

4 Section through the left popliteal lymph node from the same animal represented by figure 3. × 10.5. *sn*, secondary lymphatic nodules in *co*, cortex; *md*, medulla showing 3 large lymphopoietic centers, *lc*; *f*, adipose tissue near hilum, *hi*; *el*, efferent lymphatic; *al*, afferent lymphatic; *ca*, capsule; *pa*, perinodal areolar tissue.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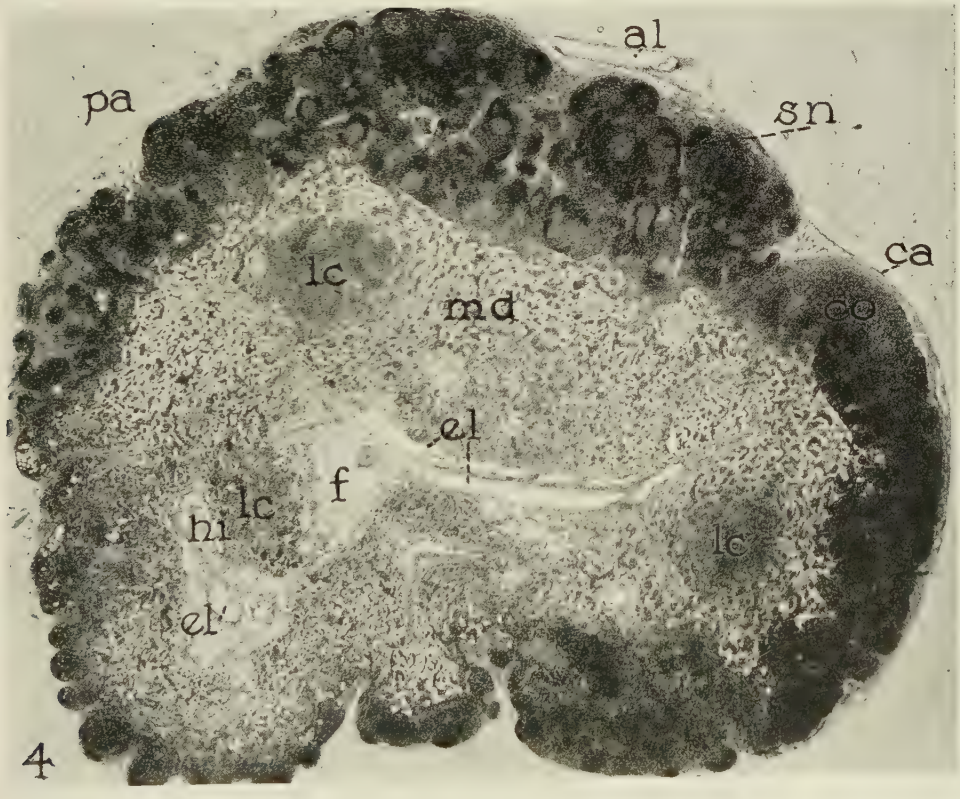
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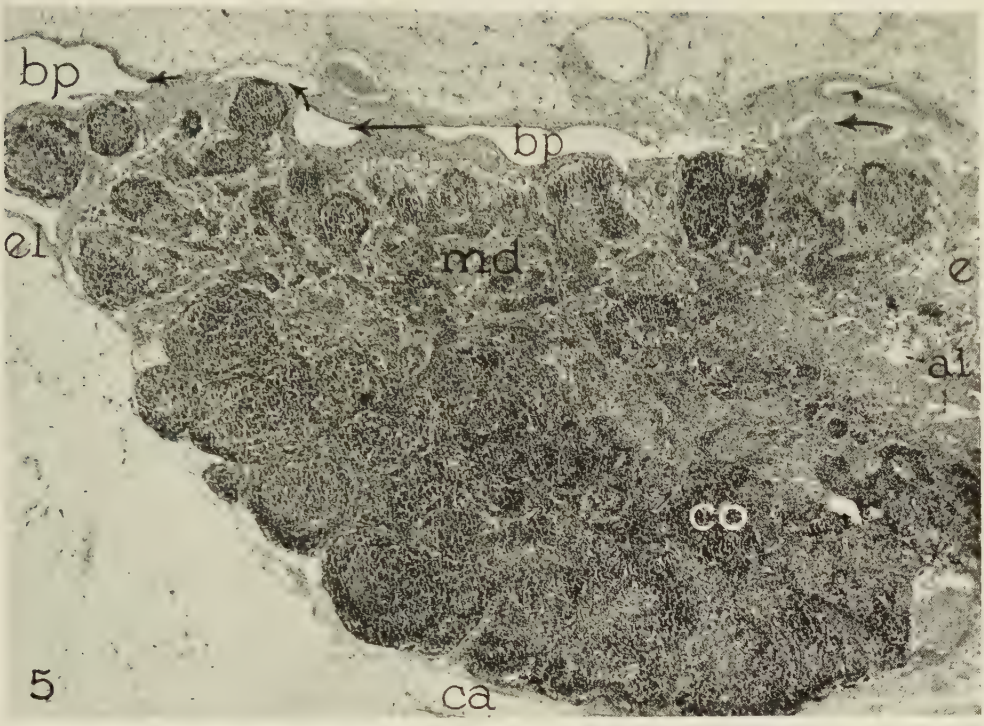
PLATE 2

EXPLANATION OF FIGURES

5 Section through the lymph node regenerated at the site of excision. (32-day-old rabbit; post-operative period, 25 days.) × 39. *co*, cortex; *md*, medulla: *al*, afferent lymphatic; *el*, efferent lymphatic emerging from hilum, *hi*; *ca*, capsule: arrows indicate course of large lymphatic by-pass, *bp*.

6 Section through the site of node excision. (14-day-old rabbit; post-operative period, 30 days.) × 398. *lr*, circumscribed lympho-reticular island containing large mesenchymal cells, *m*, mononuclear phagocytes, *ph*, with cytoplasmic inclusions and lymphocytes, *l*; *e*, scar of excision; *at*, popliteal areolar tissue; arrows indicate course of reestablished lymphatics.


bp
bp
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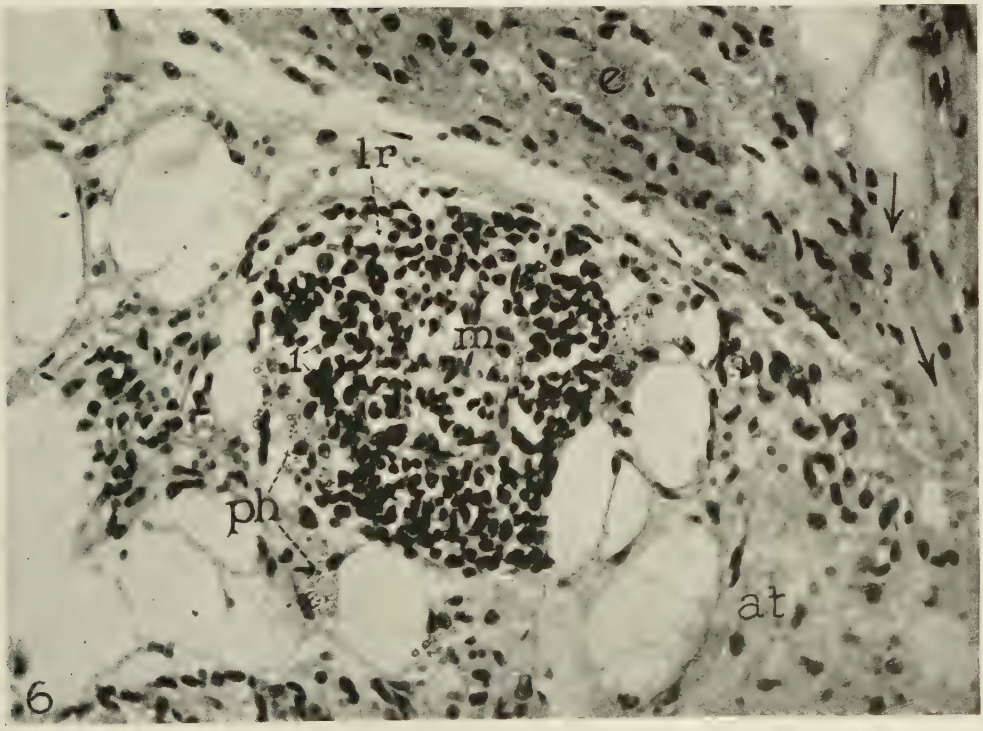
5
ca


e
lr
m
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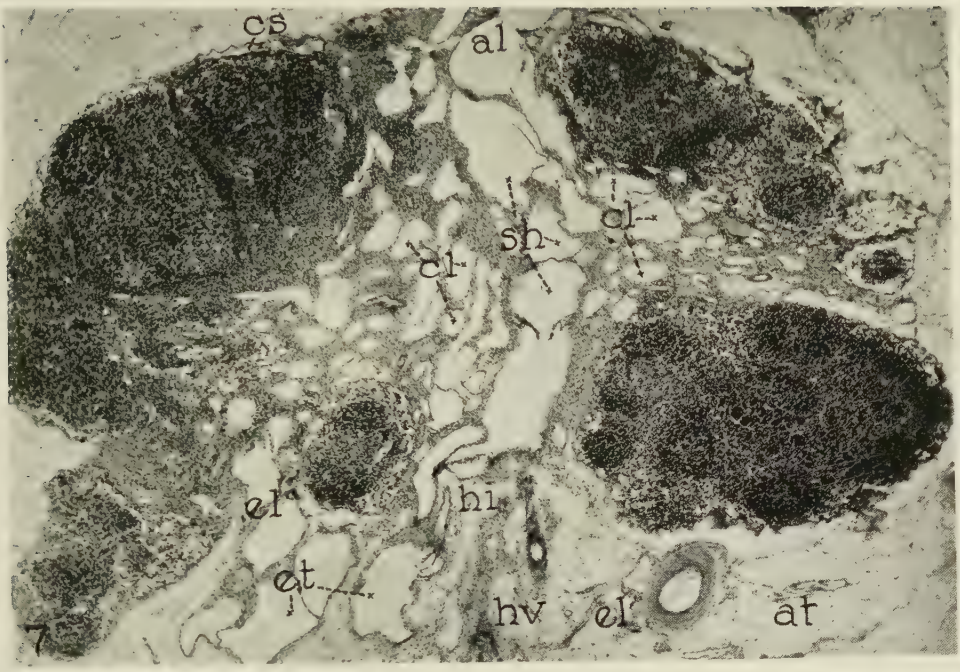
6

PLATE 3

EXPLANATION OF FIGURES

7 Section through the lymph node regenerated at the site of excision. (31-day-old rabbit; post-operative period, 31 days.) × 39. *sh*, large axial lymphatic shunt connecting afferent lymphatic, *al*, with main efferent trunk, *et*, below hilum of node, *hi*; *cs*, cortical sinus of superficial pole which connects directly with an afferent lymphatic; *cl*, collateral lymphatics which convey lymph from shunt to nodal sinuses; *el*, efferent lymphatics from node which join the main efferent trunk; *hv*, hilar blood vessels; *at*, popliteal areolar tissue.

8 Section through the site of node excision. (30-day-old rabbit; post-operative period, 31 days.) × 91. *rnd*, regenerated lymphatic nodule adjacent to large reestablished lymphatic, *rl*; *c*, peripheral cleft which shows blind bayous at the superficial and deep poles of the nodule; *bv*, chief blood vessels of nodule; *c*, scar of excision; *at*, popliteal areolar tissue.


cs
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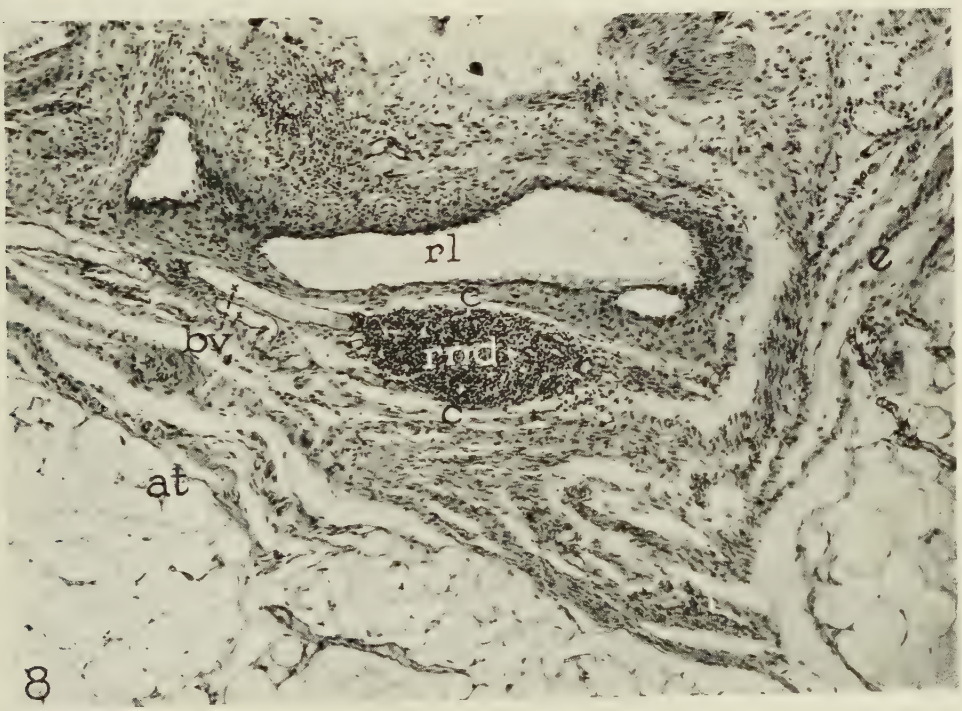
rl
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bv
prod
c
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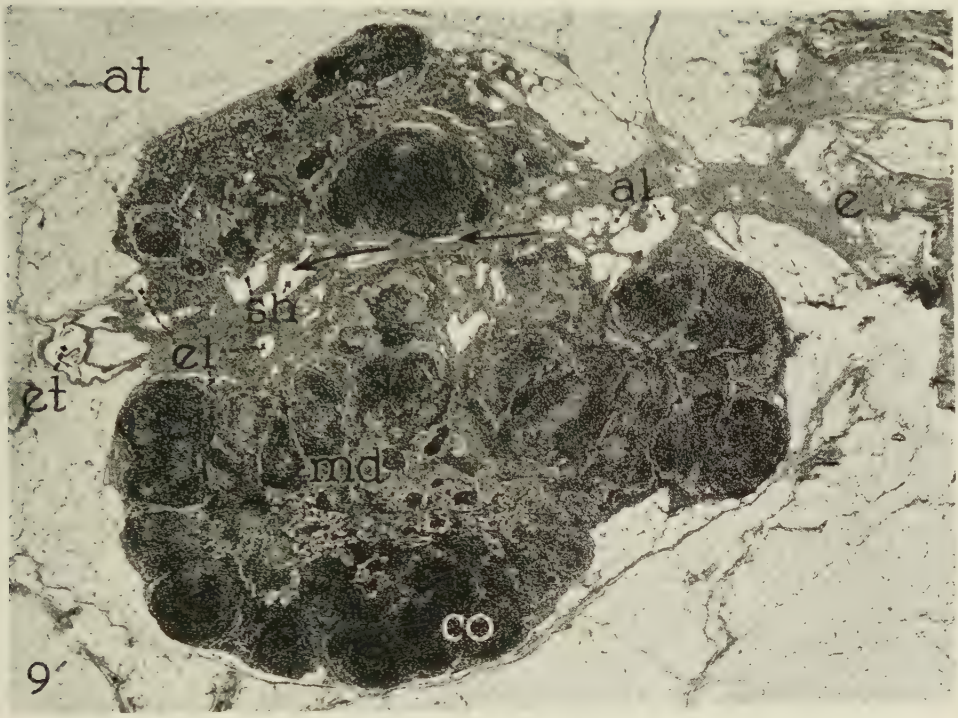
8

PLATE 4

EXPLANATION OF FIGURES

9 Section through the lymph node regenerated at the site of excision. (45-day-old rabbit; post-operative period, 31 days.) × 18. *ca*, capsule; *md*, medulla; *al*, afferent lymphatics; arrows indicate course of central shunt, *sh*, which joins efferent lymphatics of node, *el*, to form efferent trunk, *et*; *at*, popliteal areolar tissue.

10 Section through the lymph node regenerated at the site of excision. (60-day-old rabbit; post-operative period, 30 days.) × 39. *pn*, large primary lymphatic nodules of cortex; *md*, central part of medulla which contains mesenchyme-like cells with abundant protoplasmic processes; *ca*, capsule; *al*, afferent lymphatic in scar of excision, *e*; *el*, efferent lymphatic of regenerated node; *et*, efferent trunk; arrows indicate course of lymphatic by-pass; *hv*, hilar blood vessels; *at*, popliteal areolar tissue.


at
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sh
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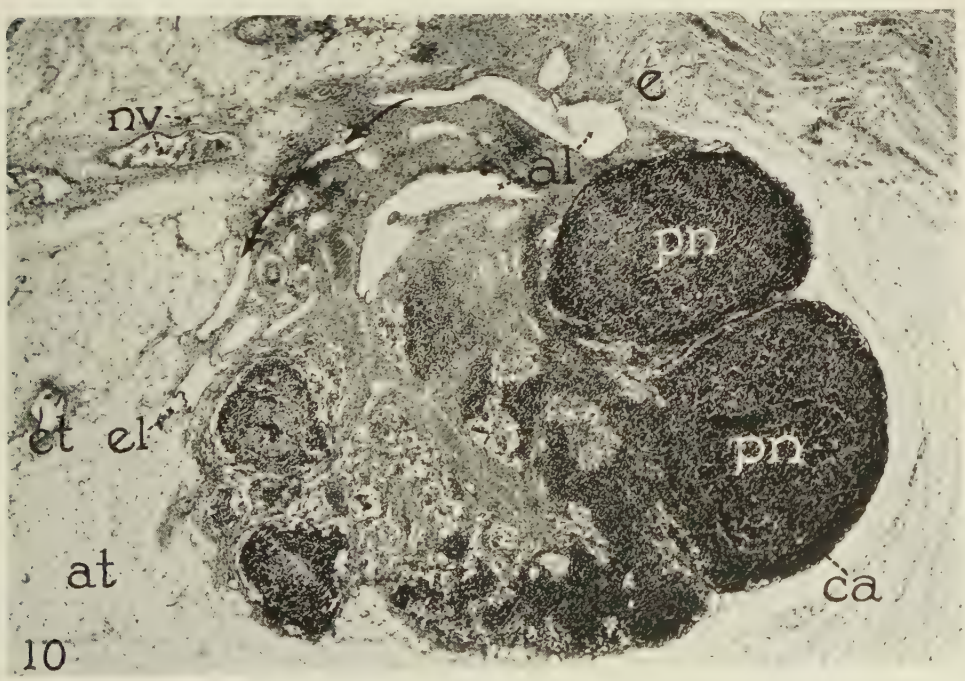
9


nv
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pn
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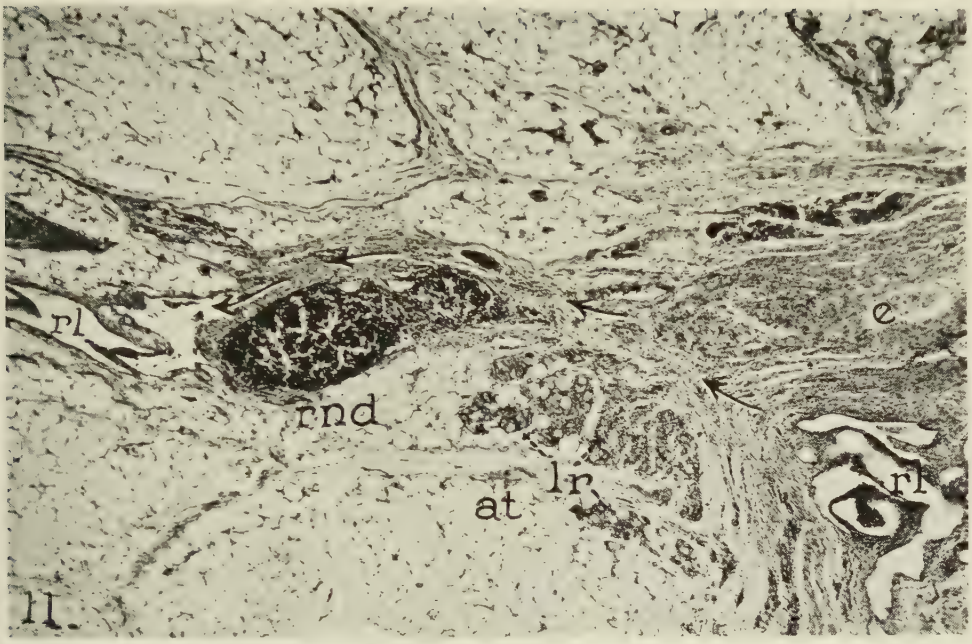
10

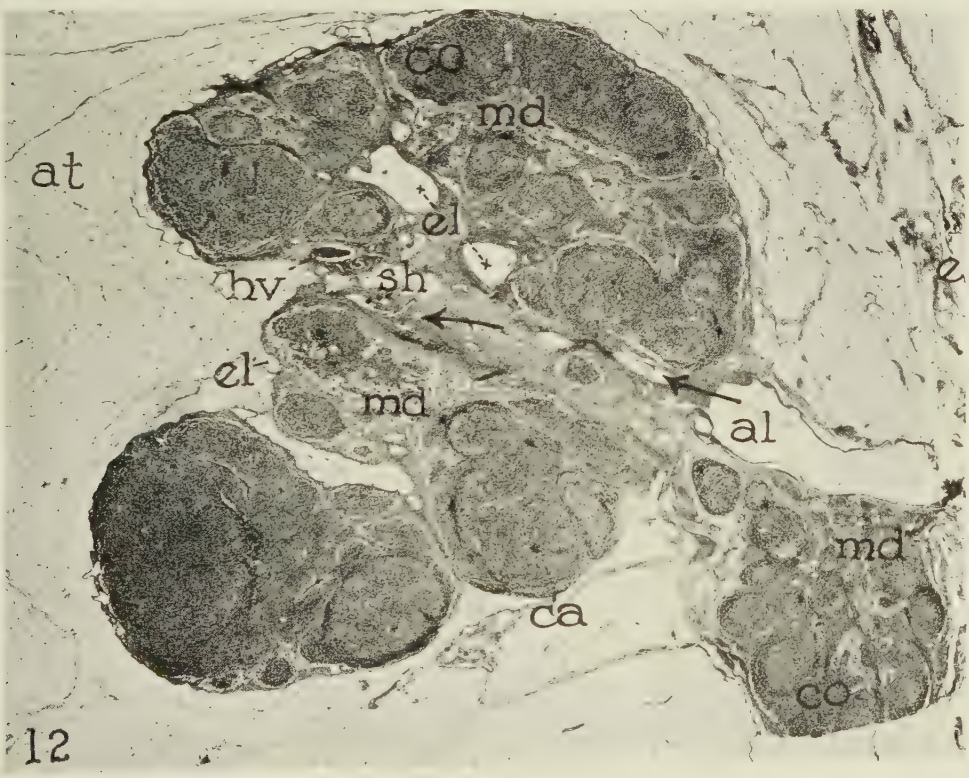
PLATE 5

EXPLANATION OF FIGURES

11 Section through the site of node excision. (60-day-old rabbit; post-operative period, 31 days.) × 42. *rnd*, regenerated lymphatic nodule near the path of reestablished lymphatics, *rl*, the course of which is indicated by arrows; clefts appearing in the nodule are artefacts; *e*, scar of excision. Note abundance of lymphoreticular foci, *lr*, in the popliteal areolar tissue, *at*.

12 Section through the lymph node regenerated at the site of excision. (95-day-old rabbit; post-operative period, 99 days.) × 20. The sinuses of cortex, *co*, and medulla, *md*, are heavily laden with injected ink; *al*, large afferent lymphatic; *el*, efferent lymphatics of regenerated node; *sh*, small terminal portion of axial lymphatic shunt; arrows indicate intra-nodal course of shunt; *hv*, hilar blood vessels; *ca*, capsule; *at*, popliteal areolar tissue; *e*, scar of excision.


rl
rnd
at
lr
e
rl
11.


at
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el
hv
sh
el
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al
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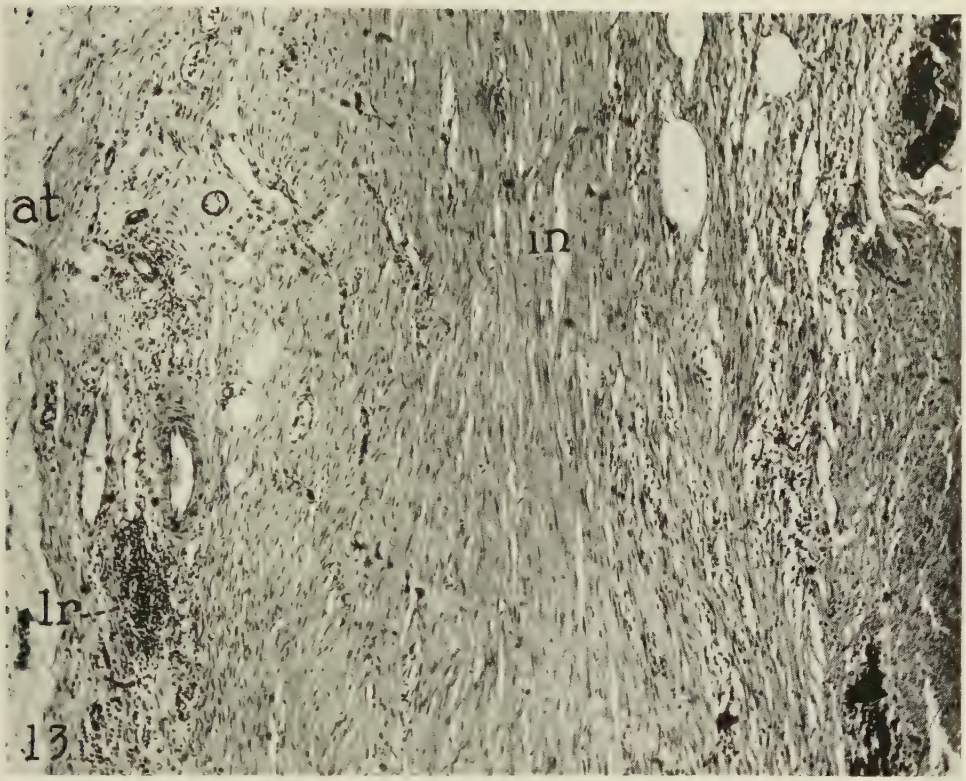
md
ca
co
12

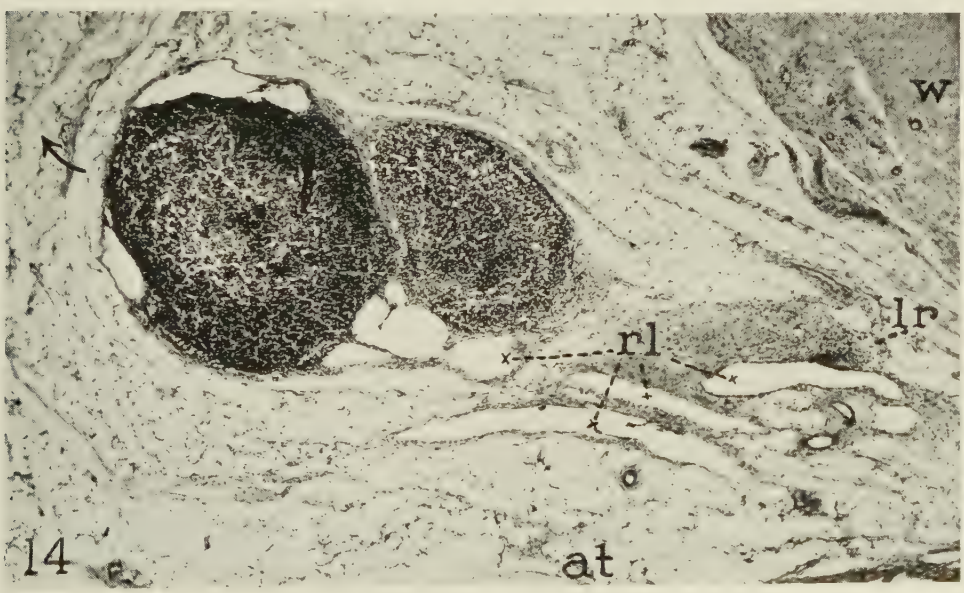
PLATE 6

EXPLANATION OF FIGURES

13 Section through the wall of abscess. × 88. *o*, outer vascular layer of granulation tissue containing lympho-reticular islands, *lr*; *in*, inner fibrous layer which lines the cavity of abscess at the right; *at*, popliteal areolar tissue.

14 Section through the large lymphatic nodule regenerated near the site of excision. (150-day-old rabbit; post-operative period, 30 days.) × 43. *rl*, re-established lymphatics by-passing wall, *w*, of central abscess and breaking up into plexiform channels around the nodule; arrows indicate the deep course taken by the lymphatics; *at*, popliteal areolar tissue containing reticular islands, *lr*.


at
o
in
lr
13


w
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rl
14
at

PLATE 7

EXPLANATION OF FIGURES

15 Section through the lymph node regenerated at the site of excision. (10-month-old rabbit; post-operative period, 217 days.) × 40. Sinuses of cortex, *co.* and medulla, *md*, congested with leucocytes and particles of injected ink; *ca*, capsule; *al*, an afferent lymphatic entering superficial pole of node; *el*, efferent lymphatic of regenerated node; *bp*, lymphatic by-pass; arrows indicate course of by-pass through popliteal areolar tissue, *at*; *e*, scar of excision.

16 Section through the residual lymph node located at the site of excision. ($2\frac{1}{2}$-year-old rabbit; post-operative period, 38 days.) × 15. *co*, cortex consisting of diffuse lymphatic mass; *md*, medulla showing extensive fibrosis especially in its central portion (light area); *ve*, vesicles containing cellular debris; *ca*, capsule of node; c^1a^1, capsule formed by condensation of perinodal areolar tissue, *pa*; *rl*, reestablished lymphatics which are not connected with the node.

co
ca
md
e
al
el
15
bp
at

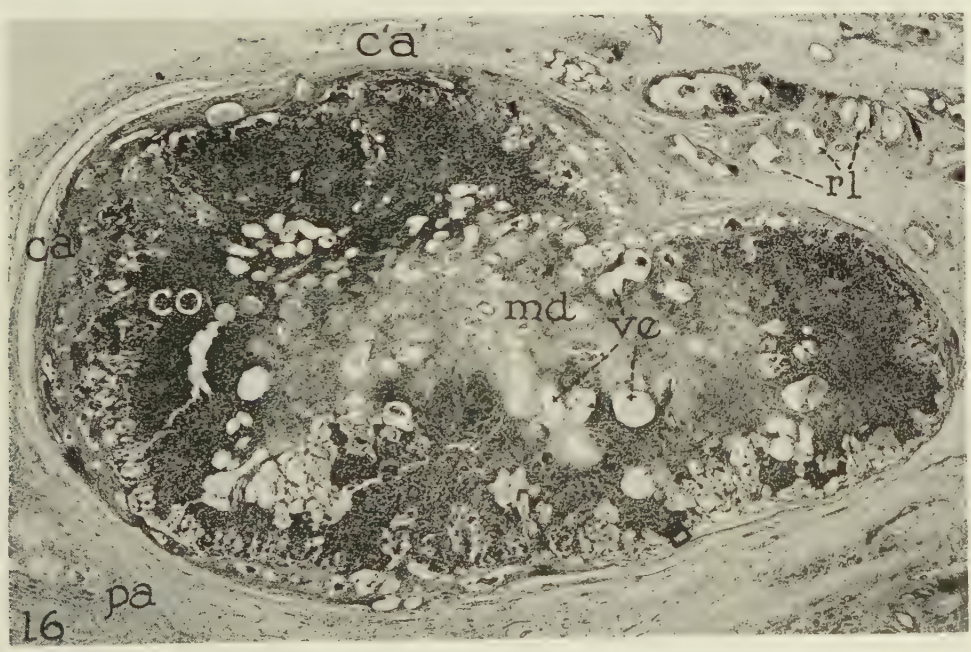
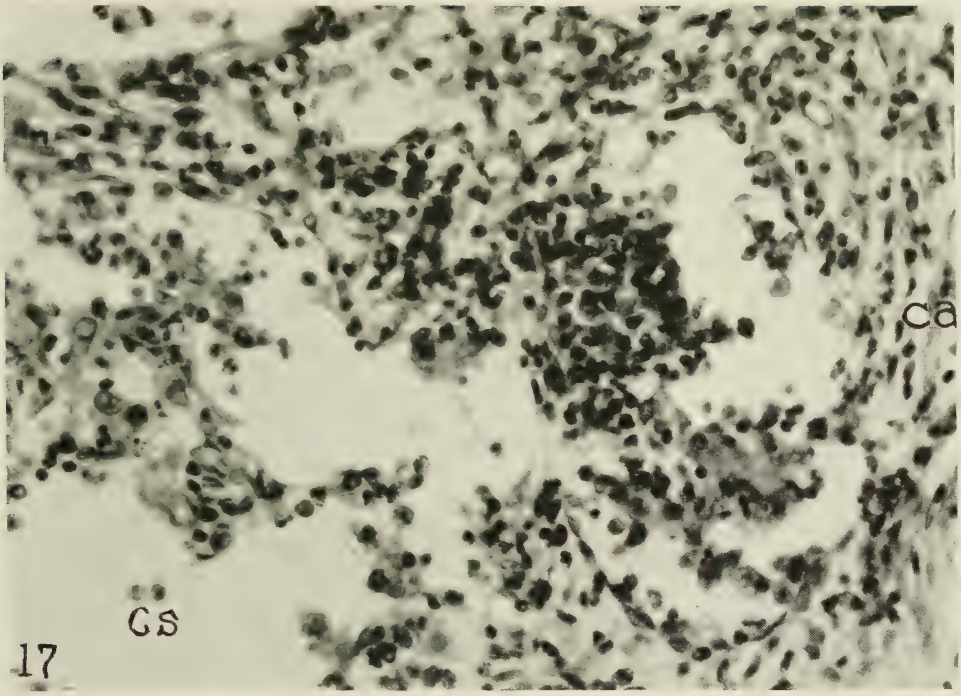

ca
rl
ca
co
md
ve
pa
16

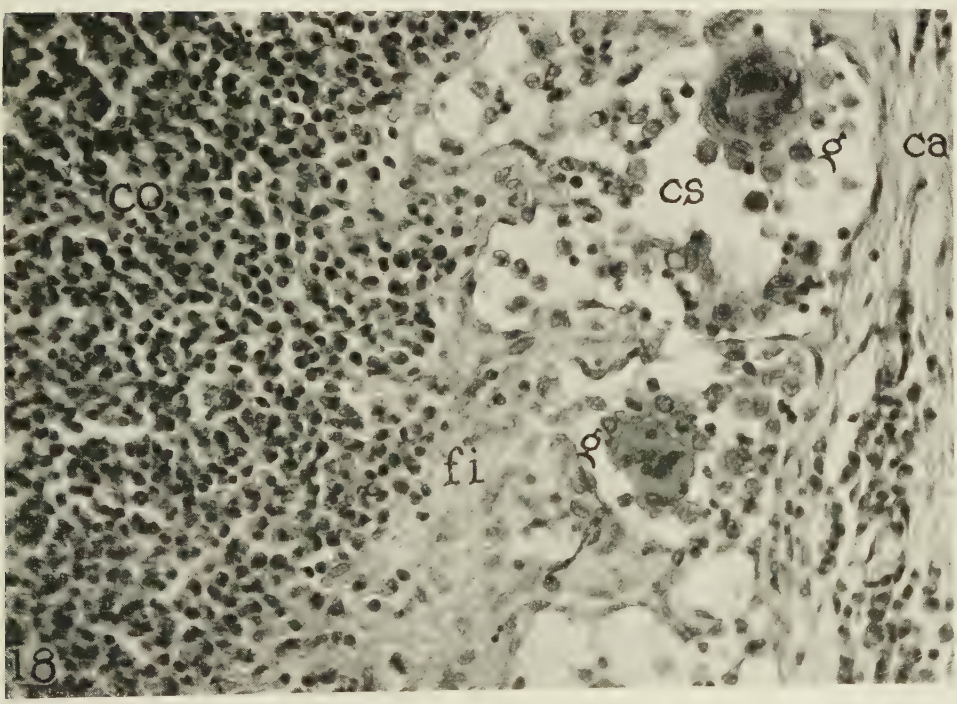
PLATE 8

EXPLANATION OF FIGURES

17 Higher magnification of the area enclosed by rectangle in figure 16. × 419. Note the abnormally wide cortical sinus, *cs*, which contains free mononuclear phagocytes; *ca*, capsule of node.

18 Area of the peripheral portion of a residual lymph node. (3½-year old rabbit; post-operative period, 46 days.) × 419. The widened cortical sinus, *cs*, contains giant cells, *g*, and numerous free and partially-detached mononuclear phagocytes, some of which show nuclear degeneration; *fi*, fibrous connective tissue extending centripetally from thickened capsule, *ca*, into cortex, *co*.


ca
cs
17


co
cs
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fi
18

PLATE 9

EXPLANATION OF FIGURE

19 Section through the cavity located at the site of excision. (45-day-old rabbit; post-operative period, 7 days.) × 20. *ac*, acidophilic coagulum containing degenerated lymphocytes; *al*, afferent lymphatics entering superficial pole of cavity; *el*, efferent lymphatic which joins remnant of original efferent trunk; *ha*, occluded stump of nodal artery transected at the time of excision. Note lymphoreticular islands, *lr*, scattered in the granulation tissue around the wall of the cavity.

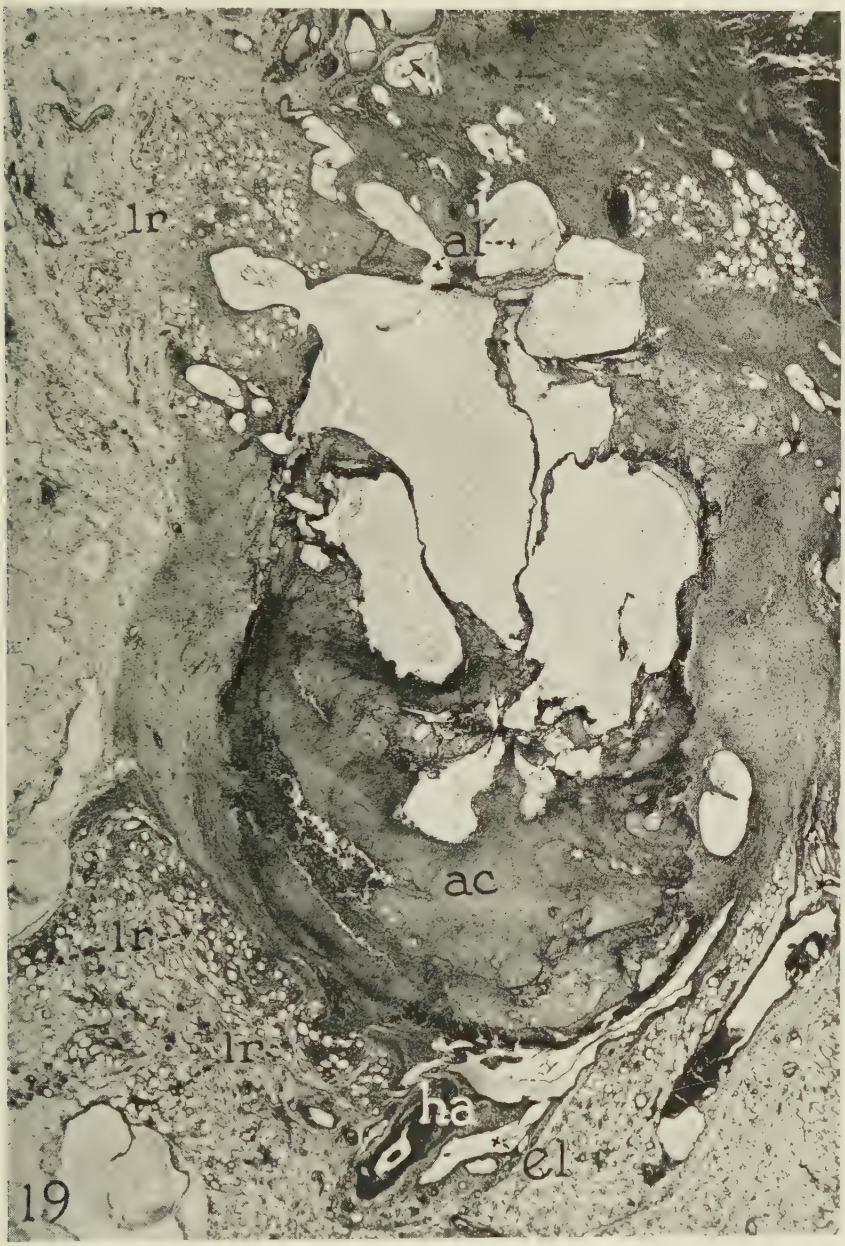
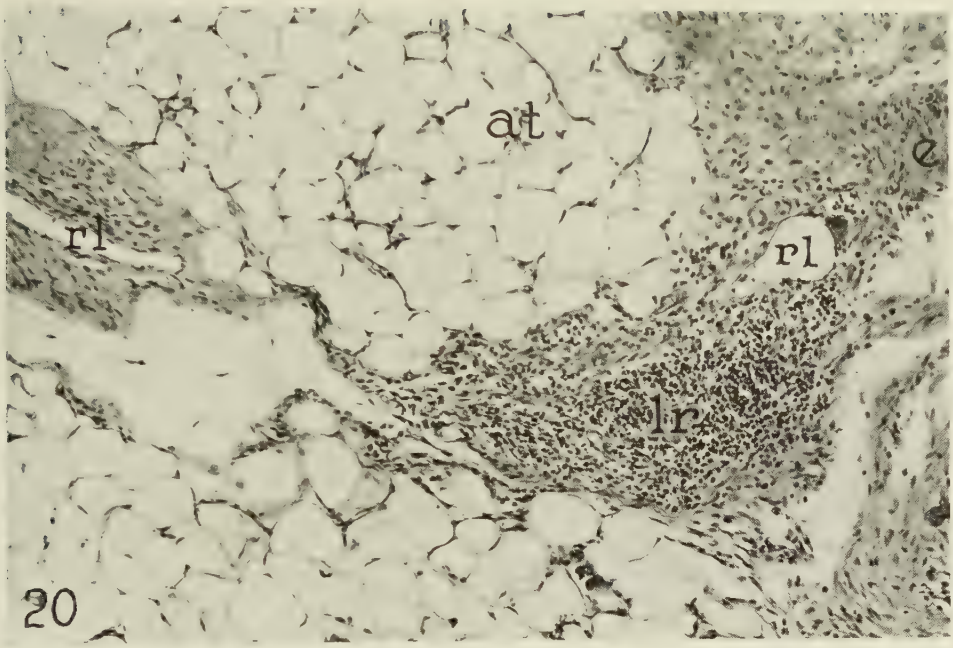

lr
al
ac
lr
lr
ha
el
19

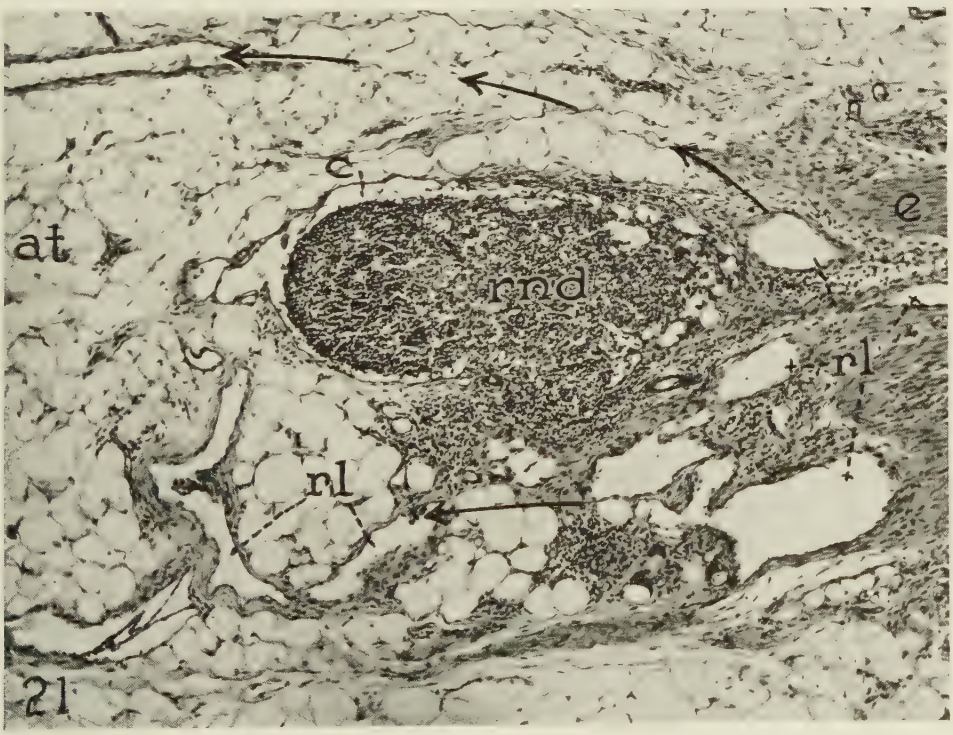
PLATE 10

EXPLANATION OF FIGURES

20 Section through the site of lymph node excision. (45-day-old rabbit; post-operative period, 14 days.) × 125. *lr*, circumscribed lympho-reticular island containing predominant number of lymphocytes; *rl*, reestablished lymphatic; *e*, scar of excision; *at*, popliteal areolar tissue.

21 Section through the site of lymph node excision. (46-day-old rabbit; post-operative period, 16 days.) × 82. *rnd*, regenerated lymphatic nodule showing discrete condensation at its deep pole, and surrounded by perinodular cleft, *c*, which ends in a blind bayou; *rl*, reestablished lymphatics passing around the lymphatic body in a course indicated by arrows; *e*, scar of excision; *at*, popliteal areolar tissue.


at
e
rl
rl
lr
20


c
e
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rnd
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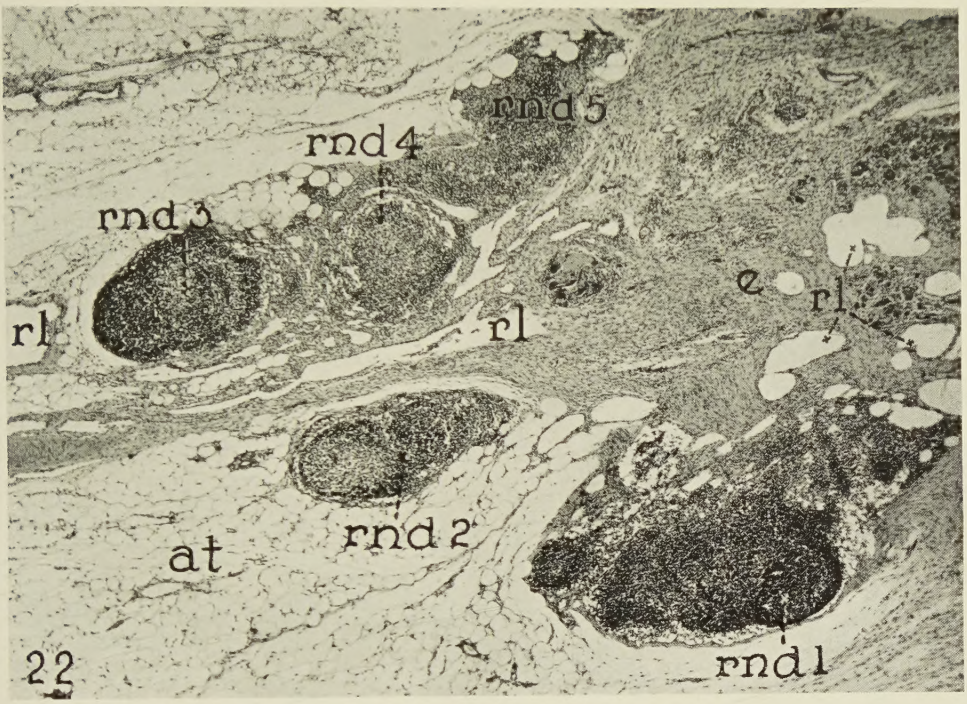
rl
21

PLATE 11

EXPLANATION OF FIGURES

22 Section through the site of lymph node excision. (46-day-old rabbit; post-operative period, 18 days.) × 35. *rnd 1*, large regenerated lymphatic nodule, the medullary sinuses of which are connected with the reestablished lymphatics through small afferent and efferent channels; *rnd 2*, *rnd 3* and *rnd 4*, regenerated lymphatic nodules which do not possess sinuses, although surrounded by plexiform branches of reestablished lymphatics; *rnd 5*, nodule consisting of unorganized lymphoreticular tissue; *rl*, reestablished lymphatics; *e*, scar of excision; *at*, popliteal areolar tissue.

23 Section through the lymph node regenerated at the site of excision. (44-day-old rabbit; post-operative period, 21 days.) × 19. *co*, cortex; *md*, medulla; *al*, afferent lymphatics passing through scar of excision, *e*; *sh*, axial lymphatic shunt; *el*, efferent lymphatics of regenerated node which join the terminal end of shunt to form the main efferent trunk, *et*; *bv*, chief blood vessels of node; *at*, popliteal areolar tissue. Note the heavy accumulation of injected ink particles in the cortical and medullary sinuses located at the right of the shunt.


rnd 5
rnd 4
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rl
rl
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e
rnd 2
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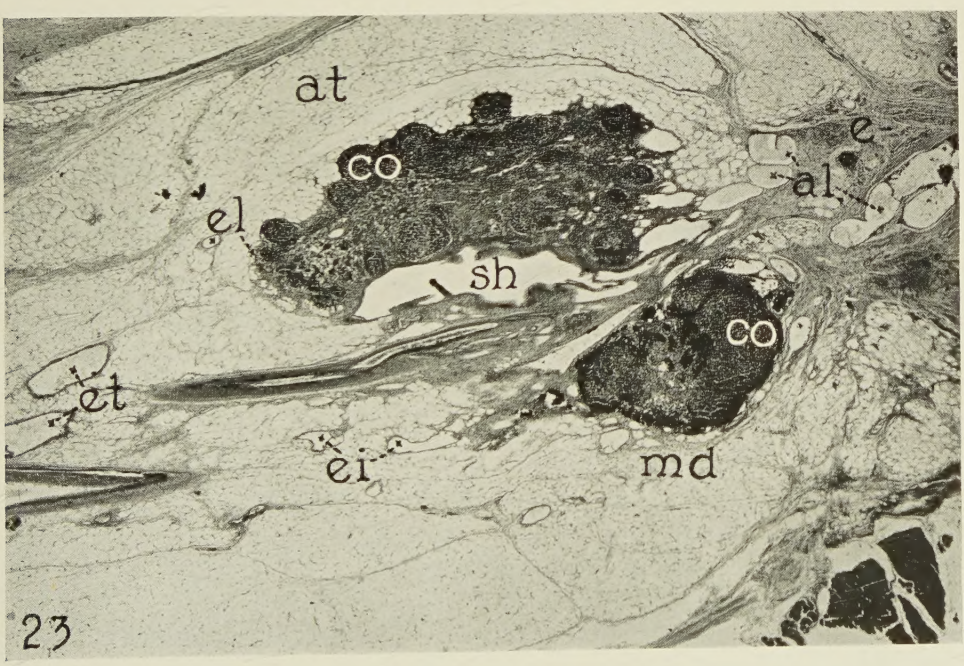
rnd 1
22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23